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(54) Title: TNFR/OPG-LIKE MOLECULES AND USES THEREOF

(57) Abstract: The present invention provides novel TNFr/OPG-like polypeptides and nucleic acid molecules encoding the same. The invention also provides vectors, host cells, antibodies, and methods for producing TNFr/OPG-like polypeptides. Also provided for are methods for the diagnosis and treatment of diseases with TNFr/OPG-like polypeptides.

TNFr/OPG-LIKE MOLECULES AND USES THEREOF

Related Application

5   The present application claims priority under 35 U.S.C.
§119 provisional patent application Serial No.60/172,306 filed
December 16, 1999.

Field of the Invention

10   The present invention relates to novel tumor necrosis
factor receptor/osteoprotegerin-like (TNFr/OPG-like)
polypeptides, and nucleic acid molecules encoding the same.
The invention also relates to vectors, host cells, selective
binding agents, such as antibodies, and methods for producing
15 TNFr/OPG-like polypeptides. Also provided for are methods for
the diagnosis and treatment of diseases associated with
TNFr/OPG-like polypeptides.

Background of the Invention

20   Technical advances in the identification, cloning,
expression and manipulation of nucleic acid molecules have
greatly accelerated the discovery of novel therapeutics based
upon deciphering of the human genome. Rapid nucleic acid
sequencing techniques can now generate sequence information at
25 unprecedented rates and, coupled with computational analyses,
allow the assembly of overlapping sequences into entire
genomes and the identification of polypeptide-encoding
regions. A comparison of a predicted amino acid sequence
against a database compilation of known amino acid sequences
30 can allow one to determine the extent of homology to
previously identified sequences and/or structural landmarks.
The cloning and expression of a polypeptide-encoding region of
a nucleic acid molecule provides a polypeptide product for

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structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides to create variants and derivatives thereof may confer advantageous properties on a product for use as a therapeutic.

5   In spite of the significant technical advances in genome research over the past decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics, or those encoding
10   polypeptides which may act as "targets" for therapeutic molecules, have still not been identified. In addition, structural and functional analyses of polypeptide products from many human genes have not been undertaken.

        Accordingly, it is an object of the invention to identify
15   novel polypeptides and nucleic acid molecules encoding the same, which have diagnostic or therapeutic benefit.

Summary of the Invention

20   The present invention relates to novel TNFr/OPG-like nucleic acid molecules and encoded polypeptides, and uses thereof.

        The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the
25   group consisting of:

        (a)   the nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

        (b)   a nucleotide sequence encoding the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;
30   (c)   a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of

the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;
and

(d)   a nucleotide sequence complementary to any of (a)
through (c).

The invention also provides for an isolated nucleic acid
molecule comprising a nucleotide sequence selected from the
group consisting of:

(a)   a nucleotide sequence encoding a polypeptide that is
at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99
percent identical to the polypeptide set forth in SEQ ID NO:
2 of SEQ ID NO: 4, wherein the polypeptide has an
activity of the encoded polypeptide set forth in SEQ ID NO: 2
or SEQ ID NO: 4 as determined using a computer program
selected from the group consisting of GAP, BLASTP, BLASTN,
FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman
algorithm;

(b)   a nucleotide sequence encoding an allelic variant or
splice variant of the nucleotide sequence set forth in SEQ ID
NOS: 1 or 3, wherein the encoded polypeptide has an activity
of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c)   a nucleotide sequence of SEQ ID NOS: 1 OR 3, (a), or
(b) encoding a polypeptide fragment of at least about 25 amino
acid residues, wherein the polypeptide has an activity of the
polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(d)   a nucleotide sequence encoding a polypeptide that
has a substitution and/or deletion of 1 to 430 amino acid
residues set forth in SEQ ID NO: 1 or 1 to 436 amino acid
residues of SEQ ID NO: 3 wherein the encoded polypeptide has
an activity of the polypeptide set forth in SEQ ID NO: 2 or
SEQ ID NO: 4;

(e)   a nucleotide sequence of SEQ ID NOS: 1 or 3 , or
(a)-(d) comprising a fragment of at least about 16
nucleotides;

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(f)   a nucleotide sequence which hybridizes under
moderately or highly stringent conditions to the complement of
any of (a)-(e), wherein the encoded polypeptide has an
activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ
5   ID NO: 4; and
(g)   a nucleotide sequence complementary to any of (a)-
(e).

The invention further provides for an isolated
10   nucleic acid molecule comprising a nucleotide sequence
selected from the group consisting of:
(a)   a nucleotide sequence encoding a polypeptide set
forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one
conservative amino acid substitution, wherein the encoded
15   polypeptide has an activity of the polypeptide set forth in
SEQ ID NO: 2 or SEQ ID NO: 4;
(b)   a nucleotide sequence encoding a polypeptide set
forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one amino
acid insertion, wherein the encoded polypeptide has an
20   activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ
ID NO: 4;
(c)   a nucleotide sequence encoding a polypeptide set
forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one amino
acid deletion, wherein the encoded polypeptide has an activity
25   of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;
(d)   a nucleotide sequence encoding a polypeptide set
forth in SEQ ID NOS: 2 or 4 which has a C- and/or N- terminal
truncation, wherein the encoded polypeptide has an activity of
the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;
30   (e)   a nucleotide sequence encoding a polypeptide set
forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one
modification selected from the group consisting of amino acid
substitutions, amino acid insertions, amino acid deletions, C-
terminal truncation, and N-terminal truncation, wherein the

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polypeptide has an activity of the encoded polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(f) a nucleotide sequence of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2or SEQ ID NO: 4; and

(h) a nucleotide sequence complementary to any of (a)-(e).

        The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the mature amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 comprising a mature amino terminus at residue 1, and optionally further comprising an amino-terminal methionine;

(b)   an amino acid sequence for an ortholog of SEQ ID NO: 2 or SEQ ID NO: 4, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c)   an amino acid sequence that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4 as determined using a computer program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm;

(d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

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(e) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or at least one of (a)-(c) wherein the polypeptide has an activity of the polypeptide set

5   forth in SEQ ID NO: 2 or SEQ ID NO: 4.

The invention further provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

10   (a)   the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(b)   the amino acid sequence set forth in SEQ ID NO: 2

15   or SEQ ID NO: 4 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c)   the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 with at least one amino acid deletion, wherein

20   the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(d)   the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set

25   forth in SEQ ID NO: 2 or SEQ ID NO: 4; and

(e)   the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and

30   N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2 or SEQ ID NO: 4.

Also provided are fusion polypeptides comprising the polypeptide sequences of (a)-(e) above of the preceding paragraphs.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules set forth herein, recombinant host cells comprising recombinant nucleic acid molecules set forth herein, and a method of producing a TNFr/OPG-like polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced. These expression vectors include baculovirus expression vectors which utilize insect cells for expression.

A transgenic non-human animal comprising a nucleic acid molecule encoding a TNFr/OPG-like polypeptide is also encompassed by the invention. The TNFr/OPG-like nucleic acid molecules are introduced into the animal in a manner that allows expression and increased levels of the TNFr/OPG-like polypeptide, which may include increased circulating levels. The transgenic non-human animal is preferably a mammal. Also provided is a transgenic non-human animal comprising a disruption in the nucleic acid molecule encoding a TNFr/OPG-like polypeptide, which will knock-out or significantly decrease expression of the TNFr/OPG-like polypeptide.

Also provided are derivatives of the TNFr/OPG-like polypeptides of the present invention.

Analogs of TNFr/OPG-like are provided for in the present invention which result from conservative and non-conservative amino acids substitutions of the TNFr/OPG-like polypeptide of SEQ ID NO: 2 or SEQ ID NO: 4. Such analogs include a TNFr/OPG-like polypeptide wherein the amino acid at position 42 of SEQ ID NO: 2 is selected from the group consisting of proline and glycine, the amino acid at position 51 of SEQ ID NO: 2 is selected from the group consisting of serine, threoinine, asparagine, glutamine, the amino acid at

position 56 of SEQ ID NO: 2 is selected from the group
consisting of phenylalanine, tryptophan, and tyrosine, the
amino acid at position 68 of SEQ ID NO: 2 is selected from the
group consisting of histadine, lysine, and arginine, the amino
5   acid at position 71 of SEQ ID NO: 2 is selected from the group
consisting of serine, cysteine, theonine, asparagine,
glutamine, the amino acid at position 84 of SEQ ID NO: 2 is
selected from the group consisting of alanine, methionine,
valine, leucine, isoleucine and norleucine or the amino acid
10   at position 87 of SEQ ID NO: 2 is selected from the group
consisting of aspartic acid or glutamic acid.

Additionally provided are selective binding agents such
as antibodies and peptides capable of specifically binding the
15   TNFr/OPG-like polypeptides of the invention. Such antibodies,
polypeptides, peptides and small molecules may be agonistic or
antagonistic.

Pharmaceutical compositions comprising the
20   nucleotides, polypeptides, or selective binding agents of the
present invention and one or more pharmaceutically acceptable
formulation agents are also encompassed by the invention. The
pharmaceutical compositions are used to provide
therapeutically effective amounts of the nucleotides or
25   polypeptides of the present invention. The invention is also
directed to methods of using the polypeptides, nucleic acid
molecules, and selective binding agents. The invention also
provides for devices to administer a TNFr/OPG-like polypeptide
encapsulated in a membrane.
30   The TNFr/OPG-like polypeptides and nucleic acid
molecules of the present invention may be used to treat,
prevent, ameliorate, diagnose and/or detect diseases and
disorders, including those recited herein. Expression
analysis in biological, cellular or tissue samples suggests

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that TNFr/OPG-like polypeptide may play a role in the diagnosis and/or treatment of the pathological conditions described herein. This expression can de detected with a diagnostic agent such as a TNFr/OPG-like polynucleotide.

5   The invention encompasses diagnosing a pathological condition or a susceptibility to a pathological condition in a subject caused by or resulting from abnormal levels of TNFr/OPG-like polypeptide comprising determining the presence or amount of expression of the TNFr/OPG-like polypeptide in a

10   sample; and comparing the level of said polypeptide in a biological, tissue or cellular sample from either normal subjects or the subject at an earlier time, wherein susceptibility to a pathological condition is based on the presence or amount of expression of the polypeptide.

15   The present invention also provides a method of assaying test molecules to identify a test molecule which binds to a TNFr/OPG-like polypeptide. The method comprises contacting a TNFr/OPG-like polypeptide with a test molecule and to determine the extent of binding of the test molecule to

20   the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of a TNFr/OPG-like polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of TNFr/OPG-like polypeptide or on the activity of

25   TNFr/OPG-like polypeptide.

         The present invention provides for methods of identifying antagonists of TNFr/OPG-like biological activity comprising contacting a small molecule compound with TNFr/OPG polypeptides and measuring TNFr/OPG-like biological activity

30   in the presence and absence of these small molecules. These small molecules can be a naturally occurring medicinal compound or derived from combinational chemical libraries. In addition, the present invention also encompasses methods which identify TNFr/OPG-like binding partners, such as cyclins.

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These methods utilize a yeast two-hybrid approach comprising a bait construct consisting of a TNFr/OPG-like polynucleotide fused to GAL4 DNA binding domain. The bait construct is used to screen a cDNA library, wherein the library consists of

5   nucleotide sequences fused to a GAL4 activation domain. Library sequences encoding TNFr/OPG-like interacting proteins can be identified by the transcriptional activation of reporter genes under the control of GAL4. See Guarente, Trend Gen., 9: 342-346 (1993); Bartel & Field, Meth. Enz., 254: 241-

10   63 (1995).

Methods of regulating expression and modulating (i.e., increasing or decreasing) levels of a TNFr/OPG-like polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule

15   encoding a TNFr/OPG-like polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of a TNFr/OPG-like polypeptide may be administered. Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described

20   herein.

In another aspect of the present invention, the TNFr/OPG-like polypeptides may be used for identifying binding partners thereof ("TNFr/OPG-like polypeptide binding partners"). Yeast two-hybrid screens have been extensively

25   used to identify and clone binding partners and receptors for proteins. (Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9583, 1991) The isolation of a TNFr/OPG-like polypeptide binding partner(s) is useful for identifying or developing novel agonists and antagonists of the TNFr/OPG-like

30   polypeptide activity.

Such agonists and antagonists include soluble TNFr/OPG cofactors, anti-TNFr/OPG selective binding agents (such as TNFr/OPG—like antibodies and derivatives thereof), small molecules, peptides or derivatives thereof capable of

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binding TNFr/OPG-like polypeptides, or antisense
oligonucleotides, any of which can be used for potentially
treating one or more diseases or disorders, including those
recited herein. These pathological conditions include, but
5   are not limited to, osteoporosis, Paget's disease,
osteomyelitis, hypercalcemia, osteopenia and osteonecrosis.

        In certain embodiments, a TNFr/OPG-like polypeptide
agonist or antagonist may be a protein, peptide,
carbohydrate, lipid, or small molecular weight molecule which
10   interacts with TNFr/OPG-like polypeptide to regulate its
activity.

Brief Description of the Figures

        Figure 1 is SEQ ID NO: 1, and sets forth the cDNA
sequence of the human TNFr/OPG-like nucleic acid molecule.

15   Figure 2 is SEQ ID NO: 3, and sets forth the cDNA
sequence of the mouse TNFr/OPG-like nucleic acid molecule.

        Figure 3 is SEQ ID NO: 2, and sets forth the amino acid
sequence of the human TNFr/OPG-like polypeptide. In this
figure, the predicted leader sequence is set forth in
20   boldface, and the predicted transmembrane region is
underlined.

        Figure 4 is SEQ ID NO: 4, and sets forth the amino acid
sequence of the mouse TNFr/OPG-like polypeptide. In this
figure, the predicted leader sequence is set forth in
25   boldface, and the predicted transmembrane region is
underlined.

        Figure 5 sets forth an overlap of the cDNA (CDR of SEQ ID
NO: 1) and predicted amino acid sequence of the human
TNFr/OPG-like polypeptide (SEQ ID NO: 2).

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Figure 6 sets forth an overlap of the cDNA (CDR of SEQ ID NO: 3) and predicted amino acid sequence of the mouse TNFr/OPG-like polypeptide (SEQ ID NO: 4).

Figure 7 and sets forth the 543 nucleotide DNA fragment
5   obtained through homology-based BLAST searches of a human genomic database (SEQ ID NO: 5). In this figure, the predicted splicing donor (GTa) and acceptor (cAG) sequences are underlined. The predicted aminpo acid sequence (SEQ ID NO: 6) of this fragment is also shown.

10   Figure 8 sets forth an amino acid sequence comparison of human osteoprotogerin (OPG; SEQ ID NO: 8) with TNFr/OPG-like polypeptide (SEQ ID NO: 7). SEQ ID NO: 7 represents amino acids 41 to 96 of SEQ ID NO: 2.

Figure 9 shows the Western blot analysis of the TNFr/OPG-
15 like Fc fusion protein that determined the TNFr/OPG-like fusion protein is cleaved by furin (left panel). The right panel displays the immunoprecipitation of full length TNFr/OPG-like recptor containing a N-terminal Flag tag from the conditioned media of TNFr/OPG-like-Fc fusion protein
20 overexpressing 293-T cells.

Figure 10 shows the flow cytometry studies performed on 20 cells lines. This analysis determined TNFr/OPG-like receptor extracellular domain binds to to Wehi-3 cells.

Figure 11 shows Northern blot analysis detecting
25 expression of TNFr/OPG-like mRNA in various tissues.

DETAILED DESCRIPTION OF THE INVENTION

The section headings used herein are for organizational purposes only and are not to be construed as limiting the
30 subject matter described therein. All references cited in

this application are expressly incorporated by reference
herein.

Definitions

5   The term "TNFr/OPG-like nucleic acid molecule" or
"polynucleotide" refers to a nucleic acid molecule comprising
or consisiting of a nucleotide sequence as set forth in either
SEQ ID NOS: 1 or 3, a nucleotide sequence encoding the
polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO:
10   4, a nucleotide sequence of a DNA insert in ATCC deposit no.
PTA-1758 and nucleic acid molecules as defined herein.
Related nucleic acid molecules include a nucleotide sequence
that is at least about 70 percent identical to the nucleotide
sequence as shown in either SEQ ID NOS: 1 or 3, or comprise or
15   consist essentially of a nucleotide sequence encoding a
polypeptide that is at least about 70 percent identical to the
polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO
4. In preferred embodiments, the nucleotide sequences are
about 75 percent, or about 80 percent, or about 85 percent, or
20   about 90 percent, or about 95, 96, 97, 98, or 99 percent
identical to the nuclectide sequence as shown in either SEQ ID
NOS: 1 or 3, or the nucleotide sequences encode a polypeptide
that is about 75 percent, or about 80 percent, or about 85
percent, or about 90 percent, or about 95, 96, 97, 98, or 99
25 _ percent identical to the polypeptide sequence as set forth in
either SEQ ID NO: 2 or SEQ ID NO: 4.

        Related nucleic acid molecules also include fragments of
the TNFr/OPG-like nucleic acid molecules which fragments
contain at least about 10 contiguous nucleotides, or about 15,
30   or about 20, or about 25, or about 50, or about 75, or about
100, or greater than about 100 contiguous nucleotides of a
TNFr/OPG-like nucleic acid molecule of either SEQ ID NOS: 1 or
3. Related nucleic acid molecules also include fragments of
the above TNFr/OPG-like nucleic acid molecules which encode a

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polypeptide of at least about 25 amino acid residues, or about
50, or about 75, or about 100, or greater than about 100 amino
acid residues of the TNFr/OPG-like polypeptide of either SEQ
ID NO: 2 or SEQ ID NO: 4. Related nucleic acid molecules also

5   include a nucleotide sequence encoding a polypeptide
comprising or consisting essentially of a substitution,
modification, addition and/or a deletion of one or more amino
acid residues compared to the polypeptide set forth in either
SEQ ID NO: 2 or SEQ ID NO: 4. In addition, related TNFr/OPG-

10 like nucleic acid molecules include those molecules which
comprise nucleotide sequences which hybridize under moderately
or highly stringent conditions as defined herein with the
fully complementary sequence of any of the TNFr/OPG-like
nucleic acid molecules of either SEQ ID NOS: 1 or 3. In

15 preferred embodiments, the related nucleic acid molecules
comprise sequences which hybridize under moderately or highly
stringent conditions with a complement of a molecule having a
sequence as shown in either SEQ ID NOS: 1 or 3, or of a
molecule encoding a polypeptide, which polypeptide comprises

20 the sequence as shown in either SEQ ID NO: 2 or SEQ ID NO: 4
or of a nucleic acid fragment as defined herein, or of a
nucleic acid fragment encoding a polypeptide as defined
herein. It is also understood that related nucleic acid
molecules include allelic or splice variants of a TNFr/OPG-

25 like nucleic acid molecule of either SEQ ID NOS: 1 or 3, and
include sequences which are complementary to any of the above
nucleotide sequences. The related encoded polypeptides
possess at least one activity of the polypeptide depicted in
either SEQ ID NO: 2 or SEQ ID NO: 4.

30   The term "isolated nucleic acid molecule" refers to a
nucleic acid molecule of the invention that (1) has been
separated from at least about 50 percent of proteins, lipids,
carbohydrates or other materials with which it is naturally
found when total DNA is isolated from the source cells, (2) is

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not linked to all or a portion of a polynucleotide to which
the "isolated nucleic acid molecule" is linked in nature, (3)
is operably linked to a polynucleotide which it is not linked
to in nature, or (4) does not occur in nature as part of a
5   larger polynucleotide sequence. Preferably, the isolated
nucleic acid molecule of the present invention is
substantially free from any other contaminating nucleic acid
molecule(s) or other contaminants that are found in its
natural environment that would interfere with its use in
10   polypeptide production or its therapeutic, diagnostic,
prophylactic or research use.

A "nucleic acid sequence" or "nucleic acid
molecule" refers to as used herein refers to a DNA or RNA
sequence. The term encompasses molecules formed from any of
15   the known base analogs of DNA and RNA such as, but not limited
to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-
cytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil,
5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-
thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil,
20   inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-
methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-
dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-
methylcytosine, 5-methylcytosine, N6-methyladenine, 7-
methylguanine, 5-methylaminome-thyluracil, 5-
25   methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5' -
methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-
N6-isopentenyladenine, uracil-5-oxyacetic acid methylester,
uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil,
queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil,
30   4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid
methylester, uracil-5-oxyacetic acid, pseudouracil, queosine,
2-thiocytosine, and 2,6-diaminopurine.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence

5   operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking

10   sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably

15   linked" to the coding sequence.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by

20   man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "allelic variant" refers to one of several possible naturally occurring alternate forms of a gene

25.   occupying a given locus on a chromosome of an organism or a population of organisms.

The term "TNFr/OPG-like splice variant" refers to a nucleic acid molecule, usually RNA, which is generated by alternative processing of intron sequences in an RNA

30   transcript of TNFr/OPG-like polypeptide amino acid sequences as set forth in SEQ ID NOS: 2 or 4.

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The term "expression vector" refers to a vector which is suitable for use in a host cell and contains nucleic acid sequences which direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression

5 includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a

10 nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

15 The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

The term "host cell".....

The term "transformation" as used herein refers to a

20 change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may

25 recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the

30 division of the cell.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced

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inside the cell membrane. A number of transfection techniques
are well known in the art and are disclosed herein. See, for
example, Graham et al., Virology, 52:456 (1973); Sambrook et
al., Molecular Cloning, a laboratory Manual, Cold Spring
5   Harbor Laboratories (New York, 1989); Davis et al., Basic
Methods in Molecular Biology, Elsevier, 1986; and Chu et al.,
Gene, 13:197 (1981). Such techniques can be used to introduce
one or more exogenous DNA moieties into suitable host cells.

The term "transduction" is used to refer to the transfer
10   of genes from one bacterium to another, usually by a phage.
"Transduction" also refers to the acquisition and transfer of
eukaryotic cellular sequences by retroviruses.

The term "host cell" is used to refer to a cell which has
been transformed, or is capable of being transformed, by a
15   vector bearing a selected gene of interest which is then
expressed by the cell. The term includes the progeny of the
parent cell, whether or not the progeny is identical in
morphology or in genetic make-up to the original parent, so
long as the selected gene is present.

20   The term "highly stringent conditions" refers to those
conditions that are designed to permit hybridization of DNA
strands whose sequences are highly complementary, and to
exclude hybridization of significantly mismatched DNAs.
Hybridization stringency is principally determined by
25   temperature, ionic strength, and the concentration of
denaturing agents such as formamide. Examples of "highly
stringent conditions" for hybridization and washing are 0.015M
sodium chloride, 0.0015M sodium citrate at 65-68°C or 0.015M
sodium chloride, 0.0015M sodium citrate, and 50% formamide at
30   42°C. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A
Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory,
(Cold Spring Harbor, N.Y. 1989); Anderson et al., Nucleic Acid

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Hybridisation: a practical approach, Ch. 4, IRL Press Limited
(Oxford, England).

More stringent conditions (such as higher temperature,
lower ionic strength, higher formamide, or other denaturing
5   agent) may also be used, however, the rate of hybridization
will be affected. Other agents may be included in the
hybridization and washing buffers for the purpose of reducing
non-specific and/or background hybridization. Examples are
0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1%
10   sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO$_4$
or (SDS), ficoll, Denhardt's solution, sonicated salmon sperm
DNA (or other non-complementary DNA), and dextran sulfate,
although other suitable agents can also be used. The
concentration and types of these additives can be changed
15   without substantially affecting the stringency of the
hybridization conditions. Hybridization experiments are
usually carried out at pH 6.8-7.4, however, at typical ionic
strength conditions, the rate of hybridization is nearly
independent of pH. See Anderson et al., Nucleic Acid
20   Hybridisation: a Practical Approach, Ch. 4, IRL Press Limited
(Oxford, England).

Factors affecting the stability of a DNA duplex include
base composition, length, and degree of base pair mismatch.
Hybridization conditions can be adjusted by one skilled in the
25   art in order to accommodate these variables and allow DNAs of
different sequence relatedness to form hybrids. The melting
temperature of a perfectly matched DNA duplex can be estimated
by the following equation:

$T_m(°C) = 81.5 + 16.6(\log[Na+]) + 0.41(\%G+C) - 600/N - 0.72(\%formamide)$

30   where N is the length of the duplex formed, [Na+] is the
molar concentration of the sodium ion in the hybridization or
washing solution, %G+C is the percentage of (guanine+cytosine)

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bases in the hybrid. For imperfectly matched hybrids, the
melting temperature is reduced by approximately 1°C for each
1% mismatch.

5   The term "moderately stringent conditions" refers to
conditions under which a DNA duplex with a greater degree of
base pair mismatching than could occur under "highly stringent
conditions" is able to form. Examples of typical "moderately
stringent conditions" are 0.015M sodium chloride, 0.0015M
sodium citrate at 50-65°C or 0.015M sodium chloride, 0.0015M
10   sodium citrate, and 20% formamide at 37-50°C. By way of
example, a "moderately stringent" condition of 50°C in 0.015 M
sodium ion will allow about a 21% mismatch.

           It will be appreciated by those skilled in the art that
there is no absolute distinction between "highly" and
15   "moderately" stringent conditions. For example, at 0.015M
sodium ion (no formamide), the melting temperature of
perfectly matched long DNA is about 71°C. With a wash at 65°C
(at the same ionic strength), this would allow for
approximately a 6% mismatch. To capture more distantly
20   related sequences, one skilled in the art can simply lower the
temperature or raise the ionic strength.

           A good estimate of the melting temperature in 1M NaCl*
for oligonucleotide probes up to about 20nt is given by:

$Tm = 2°C$ per A-T base pair + $4°C$ per G-C base pair

25   *The sodium ion concentration in 6X salt sodium citrate
(SSC) is 1M. See Suggs et al., Developmental Biology Using
Purified Genes, p. 683, Brown and Fox (eds.) (1981).

           High stringency washing conditions for oligonucleotides
are usually at a temperature of 0-5°C below the Tm of the
30   oligonucleotide in 6X SSC, 0.1% SDS.

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The term "TNFr/OPG-like polypeptide" refers to a
polypeptide comprising the amino acid sequence of either SEQ
ID NO: 2 or SEQ ID NO: 4, and related polypeptides having a
natural sequence or mutated sequence. Related polypeptides

5   include: allelic variants; splice variants; fragments;
derivatives; substitution, deletion, and insertion variants;
fusion polypeptides; and orthologs of the TNFr/OPG-like
polypeptides of either SEQ ID NO: 2 or SEQ ID NO: 4, and which
possess at least one activity of the polypeptide depicted in

10   either SEQ ID NO: 2 or SEQ ID NO: 4. TNFr/OPG-like
polypeptides may be mature polypeptides, as defined herein,
and may or may not have an amino terminal methionine residue,
depending on the method by which they are prepared.

The term "isolated polypeptide" refers to a polypeptide

15   of the present invention that (1) has been separated from at
least about 50 percent of polynucleotides, lipids,
carbohydrates or other materials with which it is naturally
found when isolated from the source cell, (2) is not linked
(by covalent or noncovalent interaction) to all or a portion

20   of a polypeptide to which the "isolated polypeptide" is linked
in nature, (3) is operably linked (by covalent or noncovalent
interaction) to a polypeptide with which it is not linked in
nature, or (4) does not occur in nature. Preferably, the
isolated polypeptide is substantially free from any other

25   contaminating polypeptides or other contaminants that are
found in its natural environment that would interfere with its
therapeutic, diagnostic, prophylactic or research use.

The term "TNFr/OPG-like polypeptide fragment" refers to a
polypeptide that comprises less than the full length amino

30   acid sequence of a TNFr/OPG-like polypeptide as set forth in
either SEQ ID NO: 2 or SEQ ID NO: 4. Such TNFr/OPG-like
fragments can be 6 amino acids or more in length, and may
arise, for example, from a truncation at the amino terminus
(with or without a leader sequence), a truncation at the

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carboxy terminus, and/or an internal deletion of one or more
residues from the amino acid sequence. TNFr/OPG-like
fragments may result from alternative RNA splicing or from *in
vivo* protease activity. Membrane-bound forms of a TNFr/OPG-

5   like polypeptide are also contemplated by the present
invention. In preferred embodiments, truncations and/or
deletions comprise about 10 amino acids, or about 20 amino
acids, or about 50 amino acids, or about 75 amino acids, or
about 100 amino acids, or more than about 100 amino acids.

10 The polypeptide fragments so produced will comprise about 25
contiguous amino acids, or about 50 amino acids, or about 75
amino acids, or about 100 amino acids, or about 150 amino
acids, or about 200 amino acids. Such TNFr/OPG-like
polypeptide fragments may optionally comprise an amino

15 terminal methionine residue. It will be appreciated that such
fragments can also be used, for example, to generate
antibodies to TNFr/OPG-like polypeptides.

The term "TNFr/OPG-like polypeptide variants" refers to
TNFr/OPG-like polypeptides which contain one or more amino

20 acid sequence substitutions, deletions, and/or additions as
compared to the TNFr/OPG-like polypeptide amino acid sequence
set forth in either SEQ ID NO: 2 or SEQ ID NO: 4. Variants
may be naturally occurring or artificially constructed. Such
TNFr/OPG-like polypeptide variants may be prepared from the

25 corresponding nucleic acid molecules encoding said variants,
which have a DNA sequence that varies accordingly from the DNA
sequences for wild type TNFr/OPG-like polypeptides as set
forth in either SEQ ID NOS: 1 or 3. In preferred embodiments,
the variants have form 1 to 3, or 1 to 5, or 1 to 10, or 1 to

30 15, or 1 to 20, or 1 to 25, or 1 to 50, or 1 to 75, or 1 to
100, or more than 100 amino acid substations, insertions,
additions and/or deletions, wherein tehh substitutions may be
conservative, or non-conservative, or any combination thereof.

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One skilled in the art will be able to determine suitable variants of the native TNFr/OPG-like polypeptide using well known techniques. For example, one may predict suitable areas of the molecule that may be changed without destroying

5 biological activity. Also, one skilled in the art will realize that even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

10 For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a TNFr/OPG-like polypeptide to such similar polypeptides. With such a comparison, one can identify

15 residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a TNFr/OPG-like polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or

20 structure of the TNFr/OPG-like like polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore,

25 even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

For predicting suitable areas of the molecule that may be

30 changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one

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skilled in the art may compare the amino acid sequence of
TNFr/OPG-like polypeptide to such similar polypeptides. After
making such a comparison, one skilled in the art can determine
residues and portions of the molecules that are conserved
5   among similar polypeptides. One skilled in the art would know
that changes in areas of the TNFr/OPG-like molecule that are
not conserved would be less likely to adversely affect the
biological activity and/or structure of a TNFr/OPG-like
polypeptide. One skilled in the art would also know that,
10   even in relatively conserved regions, one may substitute
chemically similar amino acids for the naturally occurring
residues while retaining activity (conservative amino acid
residue substitutions).

Additionally, one skilled in the art can review
15   structure-function studies identifying residues in similar
polypeptides that are important for activity or structure. In
view of such a comparison, one skilled in the art can predict
the importance of amino acid residues in a TNFr/OPG-like
polypeptide that correspond to amino acid residues that are
20   important for activity or structure in similar polypeptides.
One skilled in the art may opt for chemically similar amino
acid substitutions for such predicted important amino acid
residues of TNFr/OPG-like polypeptides.

If available, one skilled in the art can also analyze the
25   three-dimensional structure and amino acid sequence in
relation to that structure in similar polypeptides. In view
of that information, one skilled in the art may predict the
alignment of amino acid residues of TNFr/OPG-like polypeptide
with respect to its three dimensional structure. One skilled
30   in the art may choose not to make radical changes to amino
acid residues predicted to be on the surface of the protein,
since such residues may be involved in important interactions
with other molecules.

TNFr/OPG-like polypeptide analogs of the invention can be
determined by comparing the amino acid sequence of TNFr/OPG-
like polypeptide with related family members. Exemplary
TNFr/OPG-like polypeptide related family members include, but

5   are not limited to, Osteoprotegerin (OPG), and the TNF
receptor. This comparison can be accomplished by using a
Pileup alignment (Wisconsin GCG Program Package) or an
equivalent (overlapping) comparison with multiple family
members within conserved and non-conserved regions.

10   As shown in Figure 8, the predicted amino acid sequence
of human TNFr/OPG-like polypeptide (SEQ ID NO: 7; which
represent amino acid 41 to 96 of SEQ ID NO: 2) is aligned with
human OPG (SEQ ID NO: 8). Other TNFr/OPG-like polypeptide
analogs can be determined using these or other methods known

15   to those of skill in the art. These overlapping sequences
provide guidance for conservative and non-conservative amino
acids substitutions resulting in additional TNFr/OPG-like
analogs. It will be appreciated that these amino acid
substitutions can consist of naturally occurring or non-

20   naturally occurring amino acids. For example, as depicted in
Figure 8, alignment of the of related family members
indicates potential TNFr/OPG analogs may have the Pro residue
at position 42 of SEQ ID NO: 2 (position 37 on Fig. 8)
substituted with a Gly residue, the Cys residue at position 51

25   of SEQ ID NO: 2 (position 46 on Fig. 8) substituted with a
Ser, Thr, Asn or Glu residue and/or the Phe residue at
position 56 of SEQ ID NO: 2 (position 51 on Fig. 8)
substituted with a Trp, or Tyr residue. In addition,
potential TNFr/OPG analogs may have the His residue at

30   position 68 of SEQ ID NO: 2 (position 63 on Fig. 8)
substituted with a Lys or Arg residue, the Ser residue at
position 71 of SEQ ID NO: 2 (position 67 on Fig. 8)
substituted with a Cys, Thr, Asn or Gln residue, the Ala
residue at position 84 of SEQ ID NO: 2 (position 79 on Fig. 8)

substituted with a Met, Val, Leu, Ile or norleucine residue,
and/or the Asp residue at position 87 of SEQ ID NO: 2
(position 83 on Fig. 8) substituted with a Glu residue.

5   Moreover, one skilled in the art may generate test
variants containing a single amino acid substitution at each
amino acid residue. The variants could be screened using
activity assays described herein. Such variants could be used
to gather information about suitable variants. For example,
if one discovered that a change to a particular amino acid
10   residue resulted in destroyed, undesirably reduced, or
unsuitable activity, variants with such a change would be
avoided. In other words, based on information gathered from
such routine experiments, one skilled in the art can readily
determine the amino acids where further substitutions should
15   be avoided either alone or in combination with other
mutations.

      In making such changes of an equivalent nature, the
hydropathic index of amino acids may be considered. Each
amino acid has been assigned a hydropathic index on the basis
20   of their hydrophobicity and charge characteristics, these are:
isoleucine (+4.5); valine (+4.2); leucine (+3.8);
phenylalanine (+2.8); cysteine/cystine (+2.5); methionine
(+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7);
serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline
25   (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5);
aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and
arginine (-4.5).

      The importance of the hydropathic amino acid index in
conferring interactive biological function on a protein is
30   understood in the art. Kyte *et al.*, *J. Mol. Biol.*, 157:105-
131 (1982). It is known that certain amino acids may be
substituted for other amino acids having a similar hydropathic
index or score and still retain a similar biological activity.

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In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more
5   particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically' functionally equivalent protein or peptide thereby created is
10   intended for use in immunological embodiments, as in the present case.

U.S. Patent No. 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with
15   its immunogenicity and antigenicity, i.e., with a biological property of the protein. As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine
20   (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

25   In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. U.S. Patent No. 4,554,101 also
30   teaches the identification and preparation of epitopes from primary amino acid sequences on the basis of hydrophilicity. Through the methods disclosed in U.S. Patent No. 4,554,101 one of skill in the art is able to identify epitopes from within a

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given amino acid sequence.   These regions are also referred to
as "epitopic core regions".

        Desired amino acid substitutions (whether conservative or
non-conservative) can be determined by those skilled in the
5   art at the time such substitutions are desired.   For example,
amino acid substitutions can be used to identify important
residues of the TNFr/OPG-like polypeptide, or to increase or
decrease the affinity of the TNFr/OPG-like polypeptides
described herein.

10   Numerous scientific publications have been devoted to the
prediction of secondary structure, and to the identification
of epitopes, from analyses of amino acid sequences.   See Chou
et al., Biochemistry, 13(2):222-245 (1974); Chou et al.,
Biochemistry, 113(2):211-222 (1974); Chou et al., Adv.
15   Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978); Chou et
al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys.
J., 26:367-384 (1979).   Moreover, computer programs are
currently available to assist with predicting antigenic
portions and epitopic core regions of proteins.   Examples
20   include those programs based upon the Jameson-Wolf analysis
(Jameson et al., Comput. Appl. Biosci., 4(1):181-186 (1998)
and Wolf et al., Comput. Appl. Biosci., 4(1):187-191 (1988),
the program PepPlot® (Brutlag et al., CABS, 6:237-245 (1990),
and Weinberger et al., Science, 228:740-742 (1985), and other
25   new programs for protein tertiary structure prediction (Fetrow
et al., Biotechnology, 11:479-483 (1993).

        Moreover, computer programs are currently available to
assist with predicting secondary structure.   One method of
predicting secondary structure is based upon homology
30   modeling.   For example, two polypeptides or proteins which
have a sequence identity of greater than 30%, or similarity
greater than 40% often have similar structural topologies.

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The recent growth of the protein structural data base (PDB)
has provided enhanced predictability of secondary structure,
including the potential number of folds within a polypeptide's
or protein's structure. See Holm et al., Nucl. Acid. Res.,
5   27(1):244-247 (1999). It has been suggested (Brenner et al.,
Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a
limited number of folds in a given polypeptide or protein and
that once a critical number of structures have been resolved,
structural prediction will gainbecome dramatically in
10   accuracy.more accurate.

Additional methods of predicting secondary structure
include "threading""threading" (Jones, D., Curr. Opin. Struct.
Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-9
(1996)), "profile analysis"4(1):15-19 (1996)), "profile
15   analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov
et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al.,
Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and
"evolutionary linkage" (See Home, supra, "evolutionary linkage"
(See Holm, supra (1999), and Brenner, supra).

20   In preferred embodiments, the variants have from 1 to 3,
or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to
20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from
1 to 100, or more than 100 amino acid substitutions,
insertions, additions and/or deletions, wherein the
25   substitutions may be conservative, as described herein, or
non-conservative, or any combination thereof. In addition,
the variants can have additions of amino acid residues either
at the carboxy terminus or at the amino terminus (with or
without a leader sequence).

30   Preferred TNFr/OPG-like polypeptide variants include
glycosylation variants wherein the number and/or type of
glycosylation sites has been altered compared to native
TNFr/OPG-like polypeptide. In one embodiment, TNFr/OPG-like

polypeptide variants comprise a greater or a lesser number of
N-linked glycosylation sites. An N-linked glycosylation site
is characterized by the sequence: Asn-X-Ser or Thr, wherein
the amino acid residue designated as X may be any amino acid
5   residue except proline. The substitution(s) of amino acid
residues to create this sequence provides a potential new site
for the addition of an N-linked carbohydrate chain.
Alternatively, substitutions which eliminate this sequence
will remove an existing N-linked carbohydrate chain. Also
10   provided is a rearrangement of N-linked carbohydrate chains
wherein one or more N-linked glycosylation sites (typically
those that are naturally occurring) are eliminated and one or
more new N-linked sites are created. Additional preferred
TNFr/OPG-like variants include cysteine variants, wherein one
15   or more cysteine residues are deleted or substituted with
another amino acid (e.g., serine). Cysteine variants are
useful when TNFr/OPG-like polypeptides must be refolded into a
biologically active conformation such as after the isolation
of insoluble inclusion bodies. Cysteine variants generally
20   have fewer cysteine residues than the native protein, and
typically have an even number to minimize interactions
resulting from unpaired cysteines.

The term "TNFr/OPG-like fusion polypeptide" refers to a
fusion of TNFr/OPG-like polypeptide, fragment, and/or variant
25   thereof, with a heterologous peptide or polypeptide.
Heterologous peptides and polypeptides include, but are not
limited to: an epitope to allow for the detection and/or
isolation of a TNFr/OPG-like fusion polypeptide; a
transmembrane receptor protein or a portion thereof, such as
30   an extracellular domain, or a transmembrane and intracellular
domain; a ligand or a portion thereof which binds to a
transmembrane receptor protein; an enzyme or portion thereof
which is catalytically active; a polypeptide or peptide which
promotes oligomerization, such as a leucine zipper domain; a

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polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the TNFr/OPG-like polypeptide.

5   In addition, a TNFr/OPG-like polypeptide may be fused to itself or to a fragment, variant, or derivative thereof. Fusions can be made either at the amino terminus or at the carboxy terminus of a TNFr/OPG-like polypeptide. Fusions may be direct with no linker or adapter molecule or may be through
10   a linker or adapter molecule, such as one or more amino acid residues up to about 20 amino acids residues, or up to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of
15   the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

In a further embodiment of the invention, a TNFr/OPG-like polypeptide, including a fragment, variant, and/or derivative,
20   is fused to an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain known as "Fc", which links to such effector functions as complement activation and attack by phagocytic cells. An Fc has a long
25   serum half-life, whereas an Fab is short-lived. Capon *et al.*, *Nature*, 337: 525-31 (1989). When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein binding, complement fixation and perhaps even placental
30   transfer. *Id.* Table I summarizes the use of certain Fc fusions known in the art, including materials and methods applicable to the production of fused TNFr/OPG-like polypeptides.

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Table I
Fc fusion with therapeutic proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcγ2a	IL-10	anti-inflammatory; transplant rejection	Zheng et al. (1995), J. Immunol., 154: 5590-600
IgG1	TNF receptor	septic shock	Fisher et al. (1996), N. Engl. J. Med., 334: 1697-1702; Van Zee et al., (1996), J. Immunol., 156: 2221-30
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Pat. No. 5,808,029, issued September 15, 1998
IgG1	CD4 receptor	AIDS	Capon et al. (1989), Nature 337: 525-31
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill et al. (1995), Immunotech., 1: 95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614, published July 3, 1997
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cγ1	CTLA-4	autoimmune disorders	Linsley (1991), J. Exp. Med., 174:561-9

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In one example, all or a portion of the human IgG hinge,
CH2 and CH3 regions may be fused at either the N-terminus or
C-terminus of the TNFr/OPG-like polypeptides using methods
known to the skilled artisan. In another example, a portion
5   of a hinge regions and CH2 and CH3 regions may be fused. The
resulting TNFr/OPG-like Fc-fusion polypeptide may be purified
by use of a Protein A affinity column. Peptides and proteins
fused to an Fc region have been found to exhibit a
substantially greater half-life in vivo than the unfused
10   counterpart. Also, a fusion to an Fc region allows for
dimerization/multimerization of the fusion polypeptide. The
Fc region may be a naturally occurring Fc region, or may be
altered to improve certain qualities, such as therapeutic
qualities, circulation time, reduction of aggregation, etc.

15   The term "TNFr/OPG-like polypeptide derivatives" refers
to TNFr/OPG-like polypeptides, fragments, or variants, as
defined herein, that have been chemically modified. The
derivatives are modified in a manner that is different from
naturally occurring TNFr/OPG-like polypeptides, either in the
20   type or location of the molecules attached to the polypeptide.
Derivatives may further include molecules formed by the
deletion of one or more chemical groups which are naturally
attached to the TNFr/OPG-like polypeptide.

For example, the polypeptides may be modified by the
25   covalent attachment of one or more polymers, including, but
not limited to, water soluble polymers, N-linked or O-linked
carbohydrates, sugars, phosphates, and/or other such
molecules. For example, the polymer selected is typically
water soluble so that the protein to which it is attached does
30   not precipitate in an aqueous environment, such as a
physiological environment. The polymer may be of any
molecular weight, and may be branched or unbranched. Included
within the scope of suitable polymers is a mixture of

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polymers. Preferably, for therapeutic use of the end-product
preparation, the polymer will be pharmaceutically acceptable.

Suitable water soluble polymers or mixtures thereof
include, but are not limited to, polyethylene glycol (PEG),
5   monomethoxy-polyethylene glycol, dextran (such as low
molecular weight dextran, of, for example about 6 kD),
cellulose, or other carbohydrate based polymers, poly-(N-vinyl
pyrrolidone) polyethylene glycol, propylene glycol
homopolymers, a polypropylene oxide/ethylene oxide co-polymer,
10   polyoxyethylated polyols (e.g., glycerol) and polyvinyl
alcohol. Also encompassed by the present invention are
bifunctional PEG crosslinking molecules which may be used to
prepare covalently attached TNFr/OPG-like multimers.

For the acylation reactions, the polymer(s) selected
15   should have a single reactive ester group. For reductive
alkylation, the polymer(s) selected should have a single
reactive aldehyde group. A reactive aldehyde is, for example,
polyethylene glycol propionaldehyde, which is water stable, or
mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S.
20   Patent No. 5,252,714).

The pegylation of TNFr/OPG-like polypeptides may be
carried out by any of the pegylation reactions known in the
art, as described for example in the following references:
Francis et al., Focus on Growth Factors, 3:4-10 (1992); EP
25   0154316; EP 0401384 and U.S. Patent No. 4,179,337. Pegylation
may be carried out via an acylation reaction or an alkylation
reaction with a reactive polyethylene glycol molecule (or an
analogous reactive water-soluble polymer) as described herein.

Polyethylene glycol (PEG) is a water-soluble polymer
30   suitable for use herein. As used herein, the terms
"polyethylene glycol" and "PEG" are meant to encompass any of

the forms of PEG that have been used to derivatize proteins, including mono-(C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol.

     In general, chemical derivatization may be performed under any suitable conditions used to react a biologically
5   active substance with an activated polymer molecule. Methods for preparing pegylated TNFr/OPG-like polypeptides will generally comprise the steps of (a) reacting the polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby TNFr/OPG-like
10   polypeptide becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions will be determined based on known parameters and the desired result. For example, the larger the ratio of PEG:protein, the
15   greater the percentage of poly-pegylated product. In one embodiment, the TNFr/OPG-like polypeptide derivative may have a single PEG moiety at the amino terminus. See, for example, U.S. Patent No. 5,234,784.

    Generally, conditions which may be alleviated or
20   modulated by the administration of the present TNFr/OPG-like polypeptide derivative include those described herein. However, the TNFr/OPG-like polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as
25   increased or decreased half-life, as compared to the non-derivatized molecules.

    The terms "biologically active TNFr/OPG-like polypeptides", "biologically active TNFr/OPG-like polypeptide fragments", "biologically active TNFr/OPG-like polypeptide
30   variants", and "biologically active TNFr/OPG-like polypeptide derivatives" refer to TNFr/OPG-like polypeptides having at least one activity characteristic of a TNFr/OPG-like polypeptide, such as the activity of the polypeptide set forth

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in either SEQ ID NO: 2 or SEQ ID NO: 4. In general, TNFr/OPG-
like polypeptides, fragments, variants, and derivatives
thereof, will have at least one activity characteristic of a
TNFr/OPG-like polypeptide such as depicted in either SEQ ID
5 NO: 2 or SEQ ID NO: 4. In addition, a TNFr/OPG-like
polypeptide may be active as an immunogen, that is, the
polypeptide contains at least one epitope to which antibodies
may be raised.

        "Naturally occurring" or "native" when used in connection
10 with biological materials such as nucleic acid molecules,
polypeptides, host cells, and the like, refers to materials
which are found in nature and are not manipulated by man.
Similarly, "non-naturally occurring" or "non-native" as used
herein refers to a material that is not found in nature or
15 that has been structurally modified or synthesized by man.

        The term "isolated polypeptide" refers to a polypeptide
of the present invention that is free from at least one
contaminating polypeptide that is found in its natural
environment. Preferably, the isolated polypeptide is
20 substantially free from any other contaminating mammalian
polypeptides which would interfere with its therapeutic,
preventative, or diagnostic use.

        The term "ortholog" refers to a polypeptide from another
species that corresponds to TNFr/OPG-like polypeptide amino
25 acid sequence as set forth in SEQ ID NOS: 2 or 4. For
example, mouse and human TNFr/OPG-like polypeptides are
considered orthologs of each other.

        The term "mature TNFr/OPG-like polypeptide" refers to a
polypeptide lacking a leader sequence. A mature polypeptide
30 may also include other modifications such as proteolytic
processing of the amino terminus (with or without a leader
sequence) and/or the carboxy terminus, cleavage of a smaller

polypeptide from a larger precursor, N-linked and/or O-linked
glycosylation, and the like. An exemplary mature TNFr/OPG-like
polypeptide is depicted by amino acid residue __ thorough
amino acid residue   of SEQ ID NO: 2 or amino acid residue

5   through amino acid residue 'of SEQ ID NO: 4. **FILL IN BLANKS**

        The terms "effective amount" and "therapeutically
effective amount" refer to the amount of a TNFr/OPG-like
polypeptide or TNFr/OPG-like nucleic acid molecule used to
support an observable level of one or more biological

10   activities of the TNFr/OPG-like polypeptides as set forth
herein.

        The term "selective binding agent" refers to a molecule
or molecules having specificity for TNFr/OPG-like molecules.
Selective binding agents include antibodies, such as

15   polyclonal antibodies, monoclonal antibodies (mAbs), chimeric
antibodies, CDR-grafted antibodies, anti-idiotypic (anti-Id)
antibodies to antibodies that can be labeled in soluble or
bound form, as well as fragments, regions, or derivatives
thereof which are provided by known techniques, including, but

20   not limited to enzymatic cleavage, peptide synthesis, or
recombinant techniques. The anti-TNFr/OPG-like selective
binding agents of the present invention are capable, for
example, of binding portions of TNFr/OPG-like molecules that
inhibit the binding of TNF/OPG-like molecules to TNFr/OPG-like

25   receptors.

        As used herein, the terms, "specific" and "specificity"
refer to the ability of the selective binding agents to bind
to human TNFr/OPG-like polypeptides and not to human non-
TNFr/OPG-like polypeptides. It will be appreciated, however,

30   that the selective binding agents may also bind orthologs of
TNFr/OPG-like polypeptides, that is, interspecies versions of
TNFr/OPG-like polypeptides, such as mouse and rat TNFr/OPG-

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like polypeptides. A preferred embodiment relates to antibodies that are highly specific to TNFr/OPG-like polypeptides yet do not cross-react (that is, they fail to bind) with specificity to non-TNFr/OPG-like polypeptides.

5   The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, which is additionally capable of inducing an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen can have one or more
10   epitopes. The specific binding reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

15   TNFr/OPG-like polypeptides, fragments, variants, and derivatives may be used to prepare TNFr/OPG-like selective binding agents using methods known in the art. Thus, antibodies and antibody fragments that bind TNFr/OPG-like polypeptides are within the scope of the present invention.
20   Antibody fragments include those portions of the antibody which bind to an epitope on the TNFr/OPG-like polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant
25   DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions. These antibodies may be, for example, polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, human, single chain, and/or bispecific.

30

Relatedness of Nucleic Acid Molecules and/or Polypeptides

It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of SEQ ID NOS: 1 or 3, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/ora deletion of one or more amino acid residues compared to the polypeptide in SEQ ID NOS: 2 or 4.

Fragments include molecules which encode a polypeptide of at least about 25 amino acid residues, or about 50, or about 75, or about 100, or greater than about 100, amino acid residues of the polypeptide of SEQ ID NOS: 2 or 4.

In addition, related TNFr/OPG-like nucleic acid molecules include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the nucleic acid molecule of SEQ ID NOS: 1 or 3, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in SEQ ID NOS: 3 or 4, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the TNFr/OPG-like sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of TNFr/OPG-like polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein, and those regions may be used to design probes for screening.

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The term "identity" as known in the art, refers to a
relationship between the sequences of two or more polypeptide
molecules or two or more nucleic acid molecules, as determined
by comparing the sequences. In the art, "identity" also means

5   the degree of sequence relatedness between nucleic acid
molecule or polypeptide sequences, as the case may be, as
determined by the match between strings of two or more
nucleotide or two or more amino acid sequences. "Identity"
measures the percent of identical matches between the smaller

10   of two or more sequences with gap alignments (if any)
addressed by a particular mathematical model or computer
programs (i.e., "algorithms").

The term "similarity" is a related concept, but in
contrast to "identity", refers to a measure of similarity

15   which includes both identical matches and conservative
substitution matches. If two polypeptide sequences have, for
example, 10/20 identical amino acids, and the remainder are
all non-conservative substitutions, then the percent identity
and similarity would both be 50%. If in the same example,

20   there are 5 more positions where there are conservative
substitutions, then the percent identity remains 50%, but the
per cent similarity would be 75% (15/20). Therefore, in cases
where there are conservative substitutions, the degree of
similarity between two polypeptide sequences will be higher

25   than the percent identity between those two polypeptides.

In another embodiment, related nucleic acid molecules
comprise or consist of a nucleotide sequence that is about 70
percent (70%) identical to the nucleotide sequence as shown in
SEQ ID NOS: 1 or 3, or comprise or consist essentially of a

30   nucleotide sequence encoding a polypeptide that is about 70
percent (70%) identical to the polypeptide as set forth in SEQ
ID NOS: 2 or 4. In preferred embodiments, the nucleotide
sequences are about 75 percent, or about 80 percent, or about

85 percent, or about 90 percent, or about 95, 96, 97, 98, or
99 percent identical to the nucleotide sequence as shown in
SEQ ID NOS: 1 or 3, or the nucleotide sequences encode a
polypeptide that is about 75 percent, or about 80 percent, or

5   about 85 percent, or about 90 percent, or about 95, 96, 97,
98, or 99 percent identical to the polypeptide sequence as set
forth in SEQ ID NOS: 2 or 4.

       Differences in the nucleic acid sequence may result in
conservative and/or non-conservative modifications of the

10   amino acid sequence relative to the amino acid sequence of SEQ
ID NOS: 2 or 4.

       The term "conservative amino acid substitution" refers to
a substitution of a native amino acid residue with a nonnative
residue such that there is little or no effect on the polarity

15   or charge of the amino acid residue at that position. For
example, a conservative substitution results from the
replacement of a non-polar residue in a polypeptide with any
other non-polar residue. Furthermore, any native residue in
the polypeptide may also be substituted with alanine, as has

20   been previously described for "alanine scanning mutagenesis."
General rules for making amino acid substitutions are set
forth in Table II.

25

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Table II

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

5   Conservative amino acid substitutions also encompass non-
naturally occurring amino acid residues which are typically

incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties. It will be appreciated by those skilled in the

5   art the nucleic acid and polypeptide molecules described herein may be chemically synthesized as well as produced by recombinant means.

Conservative modifications to the amino acid sequence (and the corresponding modifications to the encoding

10   nucleotides) will produce TNFr/OPG-like polypeptides having functional and chemical characteristics similar to those of naturally occurring TNFr/OPG-like polypeptides. In contrast, substantial modifications in the functional and/or chemical characteristics of TNFr/OPG-like polypeptides may be

15   accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the

20   bulk of the side chain. Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;

25   4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions may involve the exchange

30   of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human TNFr/OPG-like polypeptide that are

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homologous with non-human TNFr/OPG-like polypeptides, or into
the non-homologous regions of the molecule.

Identity and similarity of related nucleic acid molecules
and polypeptides can be readily calculated by known methods.
5 Such methods include, but are not limited to, those described
in Computational Molecular Biology, Lesk, A.M., ed., Oxford
University Press, New York, 1988; Biocomputing: Informatics
and Genome Projects, Smith, D.W., ed., Academic Press, New
York, 1993; Computer Analysis of Sequence Data, Part 1,
10 Griffin, A.M., and Griffin, H.G., eds., Humana Press, New
Jersey, 1994; Sequence Analysis in Molecular Biology, von
Heinje, G., Academic Press, 1987; Sequence Analysis Primer,
Gribskov, M. and Devereux, J., eds., M. Stockton Press, New
York, 1991; and Carillo et al., SIAM J. Applied Math., 48:
15 1073 (1988).

Preferred methods to determine identity and/or similarity
are designed to give the largest match between the sequences
tested. Methods to determine identity and similarity are
described in publicly available computer programs. Preferred
20 computer program methods to determine identity and similarity
between two sequences include, but are not limited to, the GCG
program package, including GAP (Devereux et al., Nucl. Acid.
Res., 12: 387 (1984); Genetics Computer Group, University of
Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul
25 et al., J. Mol. Biol., 215:403-410 (1990)). The BLASTX
program is publicly available from the National Center for
Biotechnology Information (NCBI) and other sources (BLAST
Manual, Altschul et al. NCB/NLM/NIH Bethesda, MD 20894;
Altschul et al., supra). The well known Smith Waterman
30 algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid
sequences may result in the matching of only a short region of

the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment

5   method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be

10   determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the

15   "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the

20   algorithm. A standard comparison matrix (see Dayhoff *et al.*, Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff *et al.*, *Proc. Natl. Acad. Sci* USA, <u>89</u>: 10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

25   Preferred parameters for a polypeptide sequence comparison include the following:

30

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          Algorithm: Needleman *et al.*, *J. Mol. Biol.*, <u>48</u>, 443-453
          (1970);

          Comparison matrix: BLOSUM 62 from Henikoff *et al.*, *Proc.*
          Natl. Acad. Sci. USA, <u>89</u>: 10915-10919 (1992);

5   Gap Penalty: 12

          Gap Length Penalty: 4

          Threshold of Similarity: 0

          The GAP program is useful with the above parameters. The

10   aforementioned parameters are the default parameters for
polypeptide comparisons (along with no penalty for end gaps)
using the GAP algorithm.

          Preferred parameters for nucleic acid molecule sequence
comparisons include the following:

15   Algorithm: Needleman *et al.*, *J. Mol Biol.*, <u>48</u>: 443-453
          (1970);

          Comparison matrix: matches = +10, mismatch = 0

          Gap Penalty: 50

          Gap Length Penalty: 3

20

          The GAP program is also useful with the above parameters.
The aforementioned parameters are the default parameters for
nucleic acid molecule comparisons.

          Other exemplary algorithms, gap opening penalties, gap

25   extension penalties, comparison matrices, thresholds of
similarity, etc. may be used by those of skill in the art,
including those set forth in the Program Manual, Wisconsin
Package, Version 9, September, 1997. The particular choices
to be made will be apparent to those of skill in the art and

30   will depend on the specific comparison to be made, such as DNA
to DNA, protein to protein, protein to DNA; and additionally,
whether the comparison is between given pairs of sequences (in

which case GAP or BestFit are generally preferred) or between
one sequence and a large database of sequences (in which case
FASTA or BLASTA are preferred).

Synthesis

5   It will appreciated by those skilled in the art the
nucleic acid and polypeptide molecules described herein may be
produced by recombinant and other means.

Nucleic Acid Molecules

10   The nucleic acid molecules encode a polypeptide comparing
the amino acid sequence of a TNFr/OPG-like polypeptide can
readily be obtained in a varienty of ways including, without
limitation, chemical synthesis, cDNA or genomic library
screening, expression library screening and/or PCR
15   amplification of cDNA.

Recombinant DNA methods used herein are generally those
set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory
Manual*, Cold Spring Harbor Laboratory Press, Cold Spring
Harbor, NY (1989), and/or Ausubel *et al.*, eds., *Current
20   Protocols in Molecular Biology*, Green Publishers Inc. and
Wiley and Sons, NY (1994). The present invention provides for
nucleic acid molecules as described herein and methods for
obtaining the molecules.

A gene or cDNA encoding a TNFr/OPG-like polypeptide or
25   fragment thereof may be obtained by hybridization screening of
a genomic or cDNA library, or by PCR amplification. Where a
gene encoding the amino acid sequence of a TNFr/OPG-like
polypeptide has been identified from one species, all or a
portion of that gene may be used as a probe to identify
30   corresponding genes from other species (orthologs) or related
genes from the same species (homologs). The probes or primers

may be used to screen cDNA libraries from various tissue
sources believed to express the TNFr/OPG-like polypeptide. In
addition, part or all of a nucleic acid molecule having the
sequence as set forth in either SEQ ID NOS: 1 or 3 may be used
5   to screen a genomic library to identify and isolate a gene
encoding the amino acid sequence of a TNFr/OPG-like
polypeptide. Typically, conditions of moderate or high
stringency will be employed for screening to minimize the
number of false positives obtained from the screen.

10   Nucleic acid molecules encoding the amino acid sequence
of TNFr/OPG-like polypeptides may also be identified by
expression cloning which employs the detection of positive
clones based upon a property of the expressed protein.
Typically, nucleic acid libraries are screened by the binding
15   of an antibody or other binding partner (e.g., receptor or
ligand) to cloned proteins which are expressed and displayed
on a host cell surface. The antibody or binding partner is
modified with a detectable label to identify those cells
expressing the desired clone.

20   Recombinant expression techniques conducted in accordance
with the descriptions set forth below may be followed to
produce these polynucleotides and to express the encoded
polypeptides. For example, by inserting a nucleic acid
sequence which encodes the amino acid sequence of a TNFr/OPG-
25   like polypeptide into an appropriate vector, one skilled in
the art can readily produce large quantities of the desired
nucleotide sequence. The sequences can then be used to
generate detection probes or amplification primers.
Alternatively, a polynucleotide encoding the amino acid
30   sequence of an TNFr/OPG-like polypeptide can be inserted into
an expression vector. By introducing the expression vector
into an appropriate host, the encoded TNFr/OPG-like
polypeptide may be produced in large amounts.

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Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically

5   complementary to two separate regions of cDNA (oligonucleotides) encoding the amino acid sequence of an TNFr/OPG-like polypeptide, are then added to the cDNA along with a polymerase such as Taq polymerase, and the polymerase amplifies the cDNA region between the two primers.

10   Another means of preparing a nucleic acid molecule encoding the amino acid sequence of a TNFr/OPG-like polypeptide, including a fragment or variant, is chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al., Angew. Chem. Intl. Ed.,

15   28: 716-734 (1989). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA

20   encoding the amino acid sequence of a TNFr/OPG-like polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length

25   nucleotide sequence of a TNFr/OPG-like polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the TNFr/OPG-like polypeptide, depending on

30   whether the polypeptide produced in the host cell is designed to be secreted from that cell.

In some cases, it may be desirable to prepare nucleic acid molecules encoding TNFr/OPG-like polypeptide variants. Nucleic acid molecules encoding variants may be produced using

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site directed mutagenesis, PCR amplification, or other
appropriate methods, where the primer(s) have the desired
point mutations (see Sambrook *et al.*, *supra*, and Ausubel *et
al.*, *supra*, for descriptions of mutagenesis techniques).
5   Chemical synthesis using methods described by Engels *et al.*,
supra, may also be used to prepare such variants. Other
methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain
codons which have been altered for the optimal expression of a
10   TNFr/OPG-like polypeptide in a given host cell. Particular
codon alterations will depend upon the TNFr/OPG-like
polypeptide(s) and host cell(s) selected for expression. Such
"codon optimization" can be carried out by a variety of
methods, for example, by selecting codons which are preferred
15   for use in highly expressed genes in a given host cell.
Computer algorithms which incorporate codon frequency tables
such as "Ecohigh.cod" for codon preference of highly expressed
bacterial genes may be used and are provided by the University
of Wisconsin Package Version 9.0, Genetics Computer Group,
20   Madison, WI. Other useful codon frequency tables include
"Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod",
"Human_high.cod", "Maize_high.cod", and "Yeast_high.cod".

In other embodiments, nucleic acid molecules encode
TNFr/OPG-like variants with conservative amino acid
25   substitutions as described herein, TNFr/OPG-like variants
comprising an addition and/or a deletion of one or more N-
linked or O-linked glycosylation sites, TNFr/OPG-like variants
having deletions and/or substitutions of one or more cysteine
residues, or TNFr/OPG-like polypeptide fragments as described
30   herein. In addition, nucleic acid molecules may encode any
combination of TNFr/OPG-like variants, fragments, and fusion
polypeptides described herein.

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Vectors and Host Cells

A nucleic acid molecule encoding the amino acid sequence of a TNFr/OPG-like polypeptide is inserted into an appropriate expression vector using standard ligation techniques. The
5   vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding the amino acid sequence of a TNFr/OPG-like
10   polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems), and/or eukaryotic host cells. Selection of the host cell will depend in part on whether a TNFr/OPG-like polypeptide is to be post-translationally modified (e.g., glycosylated and/or phosphorylated). If so,
15   yeast, insect, or mammalian host cells are preferable. For a review of expression vectors, see *Meth. Enz.*, v.185, D.V. Goeddel, ed. Academic Press Inc., San Diego, CA (1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for
20   cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a
25   transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the
30   polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, i.e., an oligonucleotide molecule located at the 5'

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or 3' end of the TNFr/OPG-like polypeptide coding sequence;
the oligonucleotide sequence encodes polyHis (such as
hexaHis), or other "tag" such as FLAG, HA (hemaglutinin
Influenza virus) or *myc* for which commercially available
5   antibodies exist. This tag is typically fused to the
polypeptide upon expression of the polypeptide, and can serve
as a means for affinity purification of the TNFr/OPG-like
polypeptide from the host cell. Affinity purification can be
accomplished, for example, by column chromatography using
10   antibodies against the tag as an affinity matrix. Optionally,
the tag can subsequently be removed from the purified
TNFr/OPG-like polypeptide by various means such as using
certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same
15   species and/or strain as the host cell), heterologous (*i.e.*,
from a species other than the host cell species or strain),
hybrid (*i.e.*, a combination of flanking sequences from more
than one source), or synthetic, or the flanking sequences may
be native sequences which normally function to regulate
20   TNFr/OPG-like polypeptide expression. As such, the source of
a flanking sequence may be any prokaryotic or eukaryotic
organism, any vertebrate or invertebrate organism, or any
plant, provided that the flanking sequences is functional in,
and can be activated by, the host cell machinery.

25   The flanking sequences useful in the vectors of this
invention may be obtained by any of several methods well known
in the art. Typically, flanking sequences useful herein other
than endogenous TNFr/OPG-like gene flanking sequences will
have been previously identified by mapping and/or by
30   restriction endonuclease digestion and can thus be isolated
from the proper tissue source using the appropriate
restriction endonucleases. In some cases, the full nucleotide
sequence of a flanking sequence may be known. Here, the

flanking sequence may be synthesized using the methods
described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is
known, it may be obtained using PCR and/or by screening a
5   genomic library with suitable oligonucleotide and/or flanking
sequence fragments from the same or another species. Where
the flanking sequence is not known, a fragment of DNA
containing a flanking sequence may be isolated from a larger
piece of DNA that may contain, for example, a coding sequence
10   or even another gene or genes. Isolation may be accomplished
by restriction endonuclease digestion to produce the proper
DNA fragment followed by isolation using agarose gel
purification, Qiagen® column chromatography (Chatsworth, CA),
or other methods known to the skilled artisan. The selection
15   of suitable enzymes to accomplish this purpose will be readily
apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those
prokaryotic expression vectors purchased commercially, and the
origin aids in the amplification of the vector in a host cell.
20   Amplification of the vector to a certain copy number can, in
some cases, be important for the optimal expression of the
TNFr/OPG-like polypeptide. If the vector of choice does not
contain an origin of replication site, one may be chemically
synthesized based on a known sequence, and ligated into the
25   vector. For example, the origin of replication from the
plasmid pBR322 (Product No. 303-3s, New England Biolabs,
Beverly, MA) is suitable for most Gram-negative bacteria and
various origins (e.g., SV40, polyoma, adenovirus, vesicular
stomatitus virus (VSV) or papillomaviruses such as HPV or BPV)
30   are useful for cloning vectors in mammalian cells. Generally,
the origin of replication component is not needed for
mammalian expression vectors (for example, the SV40 origin is
often used only because it contains the early promoter).

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A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed
5   by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein
10 necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic
15 deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic
20 and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within
25 the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely
30 adapted to survive by virtue of the selection gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection

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gene and the DNA that encodes TNFr/OPG-like polypeptides. As
a result, increased quantities of TNFr/OPG-like polypeptides
are synthesized from the amplified DNA.

5   A ribosome binding site is usually necessary for
translation initiation of mRNA and is characterized by a
Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence
(eukaryotes). The element is typically located 3' to the
promoter and 5' to the coding sequence of the TNFr/OPG-like
polypeptide to be expressed. The Shine-Dalgarno sequence is
10   varied but is typically a polypurine (i.e., having a high A-G
content). Many Shine-Dalgarno sequences have been identified,
each of which can be readily synthesized using methods set
forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct a
15   TNFr/OPG-like polypeptide out of the host cell. Typically, a
nucleotide sequence encoding the signal sequence is positioned
in the coding region of the TNFr/OPG-like nucleic acid
molecule, or directly at the 5' end of the TNFr/OPG-like
polypeptide coding region. Many signal sequences have been
20   identified, and any of those that are functional in the
selected host cell may be used in conjunction with the
TNFr/OPG-like nucleic acid molecule. Therefore, a signal
sequence may be homologous (naturally occurring) or
heterologous to the TNFr/OPG-like gene or cDNA. Additionally,
25   a signal sequence may be chemically synthesized using methods
described herein. In most cases, the secretion of a TNFr/OPG-
like polypeptide from the host cell via the presence of a
signal peptide will result in the removal of the signal
peptide from the secreted TNFr/OPG-like polypeptide. The
30   signal sequence may be a component of the vector, or it may be
a part of TNFr/OPG-like nucleic acid molecule that is inserted
into the vector.

Included within the scope of this invention is the use of
either nucleotide sequence encoding a native TNFr/OPG-like

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signal sequence joined to a TNFr/OPG-like polypeptide coding
region or a nucleotide sequence encoding a a heterologous
signal sequence joined to a TNFr/OPG-like polypeptide coding
region. The heterologous signal sequence selected should be
5   one that is recognized and processed, i.e., cleaved by a
signal peptidase, by the host cell. For prokaryotic host
cells that do not recognize and process the native TNFr/OPG-
like polypeptide signal sequence, the signal sequence is
substituted by a prokaryotic signal sequence selected, for
10   example, from the group of the alkaline phosphatase,
penicillinase, or heat-stable enterotoxin II leaders. For
yeast secretion, the native TNFr/OPG-like polypeptide signal
sequence may be substituted by the yeast invertase, alpha
factor, or acid phosphatase leaders. In mammalian cell
15   expression the native signal sequence is satisfactory,
although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in
a eukaryotic host cell expression system, one may manipulate
the various presequences to improve glycosylation or yield.
20   For example, one may alter the peptidase cleavage site of a
particular signal peptide, or add presequences, which also may
affect glycosylation. The final protein product may have, in
the -1 position (relative to the first amino acid of the
mature protein) one or more additional amino acids incident to
25   expression, which may not have been totally removed. For
example, the final protein product may have one or two amino
acid residues found in the peptidase cleavage site, attached
to the N-terminus. Alternatively, use of some enzyme cleavage
sites may result in a slightly truncated form of the desired
30   TNFr/OPG-like polypeptide, if the enzyme cuts at such area
within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule
is increased by the presence of one or more introns in the
vector; this is particularly true where a polypeptide is

produced in eukaryotic host cells, especially mammalian host
cells. The introns used may be naturally occurring within the
TNFr/OPG-like gene, especially where the gene used is a full
length genomic sequence or a fragment thereof. Where the
5   intron is not naturally occurring within the gene (as for most
cDNAs), the intron(s) may be obtained from another source.
The position of the intron with respect to flanking sequences
and the TNFr/OPG-like gene is generally important, as the
intron must be transcribed to be effective. Thus, when a
10   TNFr/OPG-like cDNA molecule is being transcribed, the
preferred position for the intron is 3' to the transcription
start site, and 5' to the polyA transcription termination
sequence. Preferably, the intron or introns will be located
on one side or the other (i.e., 5' or 3') of the cDNA such
15   that it does not interrupt the coding sequence. Any intron
from any source, including any viral, prokaryotic and
eukaryotic (plant or animal) organisms, may be used to
practice this invention, provided that it is compatible with
the host cell(s) into which it is inserted. Also included
20   herein are synthetic introns. Optionally, more than one
intron may be used in the vector.

The expression and cloning vectors of the present
invention will each typically contain a promoter that is
recognized by the host organism and operably linked to the
25   molecule encoding a TNFr/OPG-like polypeptide. Promoters are
untranscribed sequences located upstream (5') to the start
codon of a structural gene (generally within about 100 to 1000
bp) that control the transcription and translation of the
structural gene. Promoters are conventionally grouped into
30   one of two classes, inducible promoters and constitutive
promoters. Inducible promoters initiate increased levels of
transcription from DNA under their control in response to some
change in culture conditions, such as the presence or absence
of a nutrient or a change in temperature. Constitutive

promoters, on the other hand, initiate continual gene product
production; that is, there is little or no control over gene
expression. A large number of promoters, recognized by a
variety of potential host cells, are well known. A suitable
5   promoter is operably linked to the DNA encoding a TNFr/OPG-
like polypeptide by removing the promoter from the source DNA
by restriction enzyme digestion and inserting the desired
promoter sequence into the vector. The native TNFr/OPG-like
gene promoter sequence may be used to direct amplification
10   and/or expression of TNFr/OPG-like nucleic acid molecule. A
heterologous promoter is preferred, however, if it permits
greater transcription and higher yields of the expressed
protein as compared to the native promoter, and if it is
compatible with the host cell system that has been selected
15   for use.

Promoters suitable for use with prokaryotic hosts include
the beta-lactamase and lactose promoter systems; alkaline
phosphatase, a tryptophan (trp) promoter system; and hybrid
promoters such as the tac promoter. Other known bacterial
20   promoters are also suitable. Their sequences have been
published, thereby enabling one skilled in the art to ligate
them to the desired DNA sequence(s), using linkers or adapters
as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well
25   known in the art. Yeast enhancers are advantageously used
with yeast promoters. Suitable promoters for use with
mammalian host cells are well known and include, but are not
limited to, those obtained from the genomes of viruses such as
polyoma virus, fowlpox virus, adenovirus (such as
30   Adenovirus 2), bovine papilloma virus, avian sarcoma virus,
cytomegalovirus (CMV), a retrovirus, hepatitis-B virus and
most preferably Simian Virus 40 (SV40). Other suitable
mammalian promoters include heterologous mammalian promoters,
e.g., heat-shock promoters and the actin promoter.

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Additional promoters which may be of interest in
controlling TNFr/OPG-like gene transcription include, but are
not limited to: the SV40 early promoter region (Bernoist and
Chambon, *Nature*, 290:304-310, 1981); the CMV promoter; the
5   promoter contained in the 3' long terminal repeat of Rous
sarcoma virus (Yamamoto *et al.*, *Cell*, 22: 787-797, 1980); the
herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl.
Acad. Sci. USA*, 78: 144-1445, 1981); the regulatory sequences
of the metallothionine gene (Brinster *et al.*, *Nature*, 296: 39-
10   42, 1982); prokaryotic expression vectors such as the beta-
lactamase promoter (Villa-Kamaroff, *et al.*, *Proc. Natl. Acad.
Sci. USA*, 75:3727-3731, 1978); or the tac promoter (DeBoer, *et
al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25, 1983). Also of
interest are the following animal transcriptional control
15   regions, which exhibit tissue specificity and have been
utilized in transgenic animals: the elastase I gene control
region which is active in pancreatic acinar cells (Swift *et
al.*, *Cell*, 38: 639-646, 1984; Ornitz *et al.*, *Cold Spring
Harbor Symp. Quant. Biol.*, 50:399-409 (1986); MacDonald,
20   *Hepatology*, 7:425-515, 1987); the insulin gene control region
which is active in pancreatic beta cells (Hanahan, *Nature*,
315:115-122, 1985); the immunoglobulin gene control region
which is active in lymphoid cells (Grosschedl *et al.*, *Cell*,
38:647-658 (1984); Adames *et al.*, *Nature*, 318:533-538 (1985);
25   Alexander *et al.*, *Mol. Cell. Biol.*, 7:1436-1444, 1987); the
mouse mammary tumor virus control region which is active in
testicular, breast, lymphoid and mast cells (Leder *et al.*,
Cell, 45:485-495, 1986); the albumin gene control region which
is active in liver (Pinkert *et al.*, *Genes and Devel.*, 1:268-
30   276, 1987); the alphafetoprotein gene control region which is
active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.*, 5:1639-
1648, 1985; Hammer *et al.*, *Science*, 235:53-58, 1987); the
alpha 1-antitrypsin gene control region which is active in the
liver (Kelsey *et al.*, *Genes and Devel.*, 1:161-171, 1987); the

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beta-globin gene control region which is active in myeloid
cells (Mogram et al., Nature, 315:338-340, 1985; Kollias et
al., Cell, 46:89-94, 1986); the myelin basic protein gene
control region which is active in oligodendrocyte cells in the
5   brain (Readhead et al., Cell, 48:703-712, 1987); the myosin
light chain-2 gene control region which is active in skeletal
muscle (Sani, Nature, 314:283-286, 1985); and the gonadotropic
releasing hormone gene control region which is active in the
hypothalamus (Mason et al., Science, 234:1372-1378, 1986).

10   An enhancer sequence may be inserted into the vector to
increase the transcription of a DNA encoding a TNFr/OPG-like
polypeptide of the present invention by higher eukaryotes.
Enhancers are cis-acting elements of DNA, usually about 10-300
bp in length, that act on the promoter to increase
15   transcription.   Enhancers are relatively orientation and
position independent. They have been found 5' and 3' to the
transcription unit. Several enhancer sequences available from
mammalian genes are known (e.g., globin, elastase, albumin,
alpha-feto-protein and insulin). Typically, however, an
20   enhancer from a virus will be used. The SV40 enhancer, the
cytomegalovirus early promoter enhancer, the polyoma enhancer,
and adenovirus enhancers are exemplary enhancing elements for
the activation of eukaryotic promoters. While an enhancer may
be spliced into the vector at a position 5' or 3' to TNFr/OPG-
25   like nucleic acid molecule, it is typically located at a site
5' from the promoter.

     Expression vectors of the invention may be constructed
from a starting vector such as a commercially available
vector. Such vectors may or may not contain all of the
30   desired flanking sequences. Where one or more of the desired
flanking sequences are not already present in the vector, they
may be individually obtained and ligated into the vector.
Methods used for obtaining each of the flanking sequences are
well known to one skilled in the art.

Preferred vectors for practicing this invention are those
which are compatible with bacterial, insect, and mammalian
host cells. Such vectors include, *inter alia*, pCRII, pCR3,
and pcDNA3.1 (Invitrogen Company, Carlsbad, CA), pBSII

5   (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison,
WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2
(Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-
alpha (PCT Publication No. WO90/14363) and pFastBacDual
(Gibco/BRL, Grand Island, NY).

10   Additional suitable vectors include, but are not limited
to, cosmids, plasmids or modified viruses, but it will be
appreciated that the vector system must be compatible with the
selected host cell. Such vectors include, but are not limited
to plasmids such as Bluescript® plasmid derivatives (a high

15   copy number ColE1-based phagemid, Stratagene Cloning Systems
Inc., La Jolla CA), PCR cloning plasmids designed for cloning
Taq-amplified PCR products (*e.g.*, TOPO™ TA Cloning® Kit,
PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA), and
mammalian, yeast, or virus vectors such as a baculovirus

20   expression system (pBacPAK plasmid derivatives, Clontech, Palo
Alto, CA). The recombinant molecules can be introduced into
host cells via transformation, transfection, infection,
electroporation, or other known techniques.

After the vector has been constructed and a nucleic acid

25   molecule encoding a TNFr/OPG-like polypeptide has been
inserted into the proper site of the vector, the completed
vector may be inserted into a suitable host cell for
amplification and/or polypeptide expression. Host cells may
be prokaryotic host cells (such as *E. coli*) or eukaryotic host

30   cells (such as a yeast cell, an insect cell, or a vertebrate
cell). The host cell, when cultured under appropriate
conditions, synthesizes a TNFr/OPG-like polypeptide which can
subsequently be collected from the culture medium (if the host

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cell secretes it into the medium) or directly from the host
cell producing it (if it is not secreted). The selection of
an appropriate host cell will depend upon various factors,
such as desired expression levels, polypeptide modifications
5   that are desirable or necessary for activity, such as
glycosylation or phosphorylation, and ease of folding into a
biologically active molecule.

A number of suitable host cells are known in the art and
many are available from the American Type Culture Collection
10   (ATCC), 10801 University Boulevard Manassas, VA 20110-2209.
Examples include, but are not limited to, mammalian cells,
such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO
DHFR-cells (Urlaub et al., Proc. Natl. Acad. Sci. USA,
97:4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T
15   cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The
selection of suitable mammalian host cells and methods for
transformation, culture, amplification, screening and product
production and purification are known in the art. Other
suitable mammalian cell lines, are the monkey COS-1 (ATCC No.
20   CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1
cell line (ATCC No. CCL70). Further exemplary mammalian host
cells include primate cell lines and rodent cell lines,
including transformed cell lines. Normal diploid cells, cell
strains derived from in vitro culture of primary tissue, as
25   well as primary explants, are also suitable. Candidate cells
may be genotypically deficient in the selection gene, or may
contain a dominantly acting selection gene. Other suitable
mammalian cell lines include but are not limited to, mouse
neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines
30   derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster
cell lines, which are available from the ATCC). Each of these
cell lines is known by and available to those skilled in the
art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (*e.g.*, HB101, (ATCC No. 33694) DH5α, DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts *et al.*, *Biotechniques*, 14:810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, 4:564-572 (1993); and Lucklow *et al.* (*J. Virol.*, 67:4566-4579 (1993). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

The transformation of an expression vector for a TNFr/OPG-like polypeptide into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

One may also use transgenic animals to express glycosylated TNFr/OPG-like polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce TNFr/OPG-

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like polypeptides, however, in general, the glycosylation
occurring in plants is different from that produced in
mammalian cells, and may result in a glycosylated product
which is not suitable for human therapeutic use.

Polypeptide Production

Host cells comprising a TNFr/OPG-like polypeptide
expression vector may be cultured using standard media well
known to the skilled artisan. The media will usually contain
all nutrients necessary for the to allow growth and survival
of the cells. Suitable media for culturing *E. coli* cells
include for example, Luria Broth (LB) and/or Terrific Broth
(TB). Suitable media for culturing eukaryotic cells include
Roswell Park Memorial Institute medium 1640 (RPMI 1640),
Minimal Essential Medium (MEM) and/or Dulbecco's Modified
Eagle Medium (DMEM), all of which may be supplemented with
serum and/or growth factors as indicated by the particular
cell line being cultured. A suitable medium for insect
cultures is Grace's medium supplemented with yeastolate,
lactalbumin hydrolysate, and/or fetal calf serum, as
necessary.

Typically, an antibiotic or other compound useful for
selective growth of transformed cells is added as a supplement
to the media. The compound to be used will be dictated by the
selectable marker element present on the plasmid with which
the host cell was transformed. For example, where the
selectable marker element is kanamycin resistance, the
compound added to the culture medium will be kanamycin. Other
compounds for selective growth include ampicillin,
tetracycline, and neomycin.

The amount of a TNFr/OPG-like polypeptide produced by a
host cell can be evaluated using standard methods known in the
art. Such methods include, without limitation, Western blot

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analysis, SDS-polyacrylamide gel electrophoresis, non-
denaturing gel electrophoresis, High Performance Liquid
Chromatography (HPLC) separation, immunoprecipitation, and/or
activity assays such as DNA binding gel shift assays.

5   If a TNFr/OPG-like polypeptide has been designed to be
secreted from the host cells, the majority of polypeptide may
be found in the cell culture medium. If however, the
TNFr/OPG-like polypeptide is not secreted from the host cells,
it will be present in the cytoplasm and/or the nucleus (for
10   eukaryotic host cells) or in the cytosol (for bacterial host
cells).

           For a TNFr/OPG-like polypeptide situated in the host cell
cytoplasm and/or the nucleus (for eukaryotic host cells) or in
the cytosol (for bacterial host cells), the host cells are
15   typically first disrupted mechanically or with a detergent to
release the intra-cellular contents into a buffered solution.
TNFr/OPG-like polypeptide can then be isolated from this
solution.

           The purification of a TNFr/OPG-like polypeptide from
20   solution can be accomplished using a variety of techniques.
If the polypeptide has been synthesized such that it contains
a tag such as Hexahistidine (TNFr/OPG-like
polypeptide/hexaHis) or other small peptide such as FLAG
(Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen,
25   Carlsbad, CA) at either its carboxyl or amino terminus, it may
essentially be purified in a one-step process by passing the
solution through an affinity column where the column matrix
has a high affinity for the tag. For example, polyhistidine
binds with great affinity and specificity to nickel, thus an
30   affinity column of nickel (such as the Qiagen" nickel columns)
can be used for purification of TNFr/OPG-like
polypeptide/polyHis. See for example, Ausubel *et al.*, eds.,

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Current Protocols in Molecular Biology, Section 10.11.8, John
Wiley & Sons, New York (1993).

Additionally, the TNFr/OPG-like polypeptide may be
purified through the use of a monoclonal antibody whih is
5   capable of specifically recognizing and binding to the
TNFr/OPG-like polypeptide.

Where a TNFr/OPG-like polypeptide is prepared without a
tag attached, and no antibodies are available, other well
known procedures for purification can be used. Such
10   procedures include, without limitation, ion exchange
chromatography, molecular sieve chromatography, High
Performance Liquid Chromatography (HPLC), native gel
electrophoresis in combination with gel elution, and
preparative isoelectric focusing ("Isoprime"
15   machine/technique, Hoefer Scientific, San Francisco, CA). In
some cases, two or more of these techniques may be combined to
achieve increased purity.

If a TNFr/OPG-like polypeptide is produced
intracellularly, the intracellular material (including
20   inclusion bodies for gram-negative bacteria) can be extracted
from the host cell using any standard technique known to the
skilled artisan. For example, the host cells can be lysed to
release the contents of the periplasm/cytoplasm by French
press, homogenization, and/or sonication followed by
25   centrifugation.

If a TNFr/OPG-like polypeptide has formed inclusion
bodies in the cytosol, the inclusion bodies can often bind to
the inner and/or outer cellular membranes and thus will be
found primarily in the pellet material after centrifugation.
30   The pellet material can then be treated at pH extremes or with
a chaotropic agent such as a detergent, guanidine, guanidine
derivatives, urea, or urea derivatives in the presence of a
reducing agent such as dithiothreitol at alkaline pH or tris

carboxyethyl phosphine at acid pH to release, break apart, and
solubilize the inclusion bodies. This solubilized TNFr/OPG-
like polypeptide in its now soluable form can then be
analyzed using gel electrophoresis, immunoprecipitation or the

5   like. If it is desired to isolate the TNFr/OPG-like
polypeptide, isolation may be accomplished using standard
methods such as those described herein and in Marston *et al.*,
Meth. Enz., 182:264-275 (1990).

In some cases, a TNFr/OPG-like polypeptide may not be

10   biologically active upon isolation. Various methods for
"refolding" or converting the polypeptide to its tertiary
structure and generating disulfide linkages, can be used to
restore biological activity. Such methods include exposing
the solubilized polypeptide to a pH usually above 7 and in the

15   presence of a particular concentration of a chaotrope. The
selection of chaotrope is very similar to the choices used for
inclusion body solubilization, but usually the chaotrope is
used at a lower concentration and is not necessarily the same
as chaotropes used for the solubilization. In most cases the

20   refolding/oxidation solution will also contain a reducing
agent or the reducing agent plus its oxidized form in a
specific ratio to generate a particular redox potential
allowing for disulfide shuffling to occur in the formation of
the protein's cysteine bridge(s). Some of the commonly used

25   redox couples include cysteine/cystamine, glutathione
(GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/
dithiane DTT, and 2-2mercaptoethanol(bME)/dithio-b(ME). A
cosolvent may be used to increase the efficiency of the
refolding, and the more common reagents used for this purpose

30   include glycerol, polyethylene glycol of various molecular
weights, arginine and the like.

If inclusion bodies are not formed to a significant
degree upon expression of a TNFr/OPG-like polypeptide, then
the polypeptide will be found primarily in the supernatant

after centrifugation of the cell homogenate. The polypeptide
may be further isolated from the supernatant using methods
such as those described herein.

Suitable procedures for purification thus include,
5   without limitation, affinity chromatography, immunoaffinity
chromatography, ion exchange chromatography, molecular sieve
chromatography, High Performance Liquid Chromatography (HPLC),
electrophoresis (including native gel electrophoresis)
followed by gel elution, and preparative isoelectric focusing
10   ("Isoprime" machine/technique, Hoefer Scientific, San
Francisco, CA). In some cases, two or more purification
techniques may be combined to achieve increased purity.

TNFr/OPG-like polypeptides, including fragments,
variants, and/or derivatives thereof may also be prepared by
15   chemical synthesis methods (such as solid phase peptide
synthesis) using techniques known in the art, such as those
set forth by Merrifield *et al.*, *J. Am. Chem. Soc.*, 85:2149
(1963), Houghten *et al.*, *Proc Natl Acad. Sci. USA*, 82:5132
(1985), and Stewart and Young, Solid Phase Peptide Synthesis,
20   Pierce Chemical Co., Rockford, IL (1984). Such polypeptides
may be synthesized with or without a methionine on the amino
terminus. Chemically synthesized TNFr/OPG-like polypeptides
may be oxidized using methods set forth in these references to
form disulfide bridges. Chemically synthesized TNFr/OPG-like
25   polypeptides are expected to have comparable biological
activity to the corresponding TNFr/OPG-like polypeptides
produced recombinantly or purified from natural sources, and
thus may be used interchangeably with a recombinant or natural
TNFr/OPG-like polypeptide.
30   Another means of obtaining a TNFr/OPG-like polypeptide is
via purification from biological samples such as source
tissues and/or fluids in which the TNFr/OPG-like polypeptide
is naturally found. Such purification can be conducted using
methods for protein purification as described herein. The

presence of the TNFr/OPG-like polypeptide during purification
may be monitored using, for example, an antibody prepared
against recombinantly produced TNFr/OPG-like polypeptide or
peptide fragments thereof.

5   A number of additional methods for producing nucleic
acids and polypeptides are known in the art, and the methods
can be used to produce polypeptides having specificity for
TNFr/OPG-like. See for example, Roberts et al., Proc. Natl.
Acad. Sci. USA, 94:12297-12303 (1997), which describes the
10   production of fusion proteins between an mRNA and its encoded
peptide. See also Roberts, R., Curr. Opin. Chem. Biol.,
3:268-273 (1999). Additionally, U.S. patentPatent No.
5,824,469 describes methods of obtaining oligonucleotides
capable of carrying out a specific biological function. The
15   procedure involves generating a heterogeneous pool of
oligonucleotides, each having a 5' randomized sequence, a
central preselected sequence, and a 3' randomized sequence.
The resulting heterogeneous pool is introduced into a
population of cells that do not exhibit the desired biological
20   function. Subpopulations of the cells are then screened for
those which exhibit a predetermined biological function. From
that subpopulation, oligonucleotides capable of carrying out
the desired biological function are isolated.
U.S. Patent Nos. 5,763,192, 5,814,476, 5,723,323, and
25   5,817,483 describe processes for producing peptides or
polypeptides. This is done by producing stochastic genes or
fragments thereof, and then introducing these genes into host
cells which produce one or more proteins encoded by the
stochastic genes. The host cells are then screened to
30   identify those clones producing peptides or polypeptides
having the desired activity.

Another method for producing peptides or polypeptides is
described in PCT/US98/20094 (WO99/15650) filed by Athersys,
Inc. Known as "Random Activation of Gene Expression for Gene

Discovery" (RAGE-GD), the process involves the activation of
endogenous gene expression or over-expression of a gene by in
situ recombination methods. For example, expression of an
endogenous gene is activated or increased by integrating a
5   regulatory sequence into the target cell which is capable of
activating expression of the gene by non-homologous or
illegitimate recombination. The target DNA is first subjected
to radiation, and a genetic promoter inserted. The promoter
eventually locates a break at the front of a gene, initiating
10   transcription of the gene. This results in expression of the
desired peptide or polypeptide.

It will be appreciated that these methods can also be
used to create comprehensive IL-17 like protein expression
libraries, which can subsequently be used for high throughput
15   phenotypic screening in a variety of assays, such as
biochemical assays, cellular assays, and whole organism assays
(e.g., plant, mouse, etc.).

Chemical Derivatives

20   Chemically modified derivatives of the TNFr/OPG-like
polypeptides may be prepared by one skilled in the art, given
the disclosures set forth hereinbelow. TNFr/OPG-like
polypeptide derivatives are modified in a manner that is
different, either in the type or location of the molecules
25   naturally attached to the polypeptide. Derivatives may
include molecules formed by the deletion of one or more
naturally-attached chemical groups. The polypeptide
comprising the amino acid sequence of SEQ ID NOS: 2 or 4, or
an TNFr/OPG-like polypeptide variant, may be modified by the
30   covalent attachment of one or more polymers. For example, the
polymer selected is typically water soluble so that the
protein to which it is attached does not precipitate in an
aqueous environment, such as a physiological environment.
Included within the scope of suitable polymers is a mixture of

polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an

5   average molecular weight of between about 2kDa to about 100kDa (the term "about""about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer is preferablyis between about

10   5kDa and5kDa, about 50kDa, more preferably between about 12kDa andto about 40kDa and most preferably between about 20kDa andto about 35kDa.

Suitable water soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked

15   carbohydrates, sugars, phosphates,carbohydrates; sugars; phosphates; polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol),glycol); monomethoxy-polyethylene glycol,glycol; dextran (such as low

20   molecular weight dextran, of, for example about 6 kD), cellulose, or otherdextran of, for example, about 6 kDa);, cellulose; or carbohydrate basedother carbohydrate-based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene

25   oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached multimers of the polypeptide comprising the amino acid sequence of SEQ ID NOS:

30   2 or 4 or an TNFr/OPG-like polypeptide variant.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides will generally comprise the steps

of (a) reacting the polypeptide with the activated polymer
molecule (such as a reactive ester or aldehyde derivative of
the polymer molecule) under conditions whereby the polypeptide
comprising the amino acid sequence of SEQ ID NOS: 2 or 4, or

5   an TNFr/OPG-like polypeptide variant becomes attached to one
or more polymer molecules, and (b) obtaining the reaction
product(s). The optimal reaction conditions will be
determined based on known parameters and the desired result.
For example, the larger the ratio of polymer

10   molecules:protein, the greater the percentage of attached
polymer molecule. In one embodiment, the TNFr/OPG-like
polypeptide derivative may have a single polymer molecule
moiety at the amino terminus. See,terminus (see, for example,
U.S. Patent No. 5,234,784).

15   The pegylation of the polypeptide may be specificallymay
be carried out by any of the pegylation reactions known in the
art, as described for example in the following references:
Francis et al., Focus on Growth Factors, 3:4-10 (1992); EP
0154316; EP 0401384 and U.S. Patent No. 4,179,337. For

20   example, pegylation may be carried out via an acylation
reaction or an alkylation reaction with a reactive
polyethylene glycol molecule (or an analogous reactive water-
soluble polymer) as described herein. For the acylation
reactions, the polymer(s) selected should have a single

25   reactive ester group. For reductive alkylation, the
polymer(s) selected should have a single reactive aldehyde
group. A reactive aldehyde is, for example, polyethylene
glycol propionaldehyde, which is water stable, or mono C1-C10
alkoxy or aryloxy derivatives thereof (see U.S. Patent No.

30   5,252,714).

    In another embodiment, TNFr/OPG-like polypeptides may be
chemically coupled to biotin, and the biotin/TNFr/OPG-like
polypeptide molecules which are conjugated are then allowed to
bind to avidin, resulting in tetravalent

avidin/biotin/TNFr/OPG-like polypeptide molecules. TNFr/OPG-
like polypeptides may also be covalently coupled to
dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting
conjugates precipitated with anti-DNP or anti-TNP-IgM to form
5   decameric conjugates with a valency of 10.

Generally, conditions which may be alleviated or
modulated by the administration of the present TNFr/OPG-like
polypeptide derivatives include those described herein for
TNFr/OPG-like polypeptides. However, the AGP-TNFr/OPG-like
10   polypeptide derivatives disclosed herein may have additional
activities, enhanced or reduced biological activity, or other
characteristics, such as increased or decreased half-life, as
compared to the non-derivatized molecules.

15   **Microarray**

It will be appreciated that DNA microarray
technology can be utilized in accordance with the present
invention. DNA microarrays are miniature, high density arrays
of nucleic acids positioned on a solid support, such as glass.
20   Each cell or element within the array has numerous copies of a
single species of DNA which acts as a target for hybridization
for its cognate mRNA. In expression profiling using DNA
microarray technology, mRNA is first extracted from a cell or
tissue sample and then converted enzymatically to
25   fluorescently labeled cDNA. This material is hybridized to
the microarray and unbound cDNA is removed by washing. The
expression of discrete genes represented on the array is then
visualized by quantitating the amount of labeled cDNA which is
specifically bound to each target DNA. In this way, the
30   expression of thousands of genes can be quantitated in a high
throughput, parallel manner from a single sample of biological
material.

This high throughput expression profiling has a broad
range of applications with respect to the TNFr/OGP-like
35   molecules of the invention, including, but not limited to: the

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identification and validation of TNFr/OGP-like disease-related
genes as targets for therapeutics; molecular toxicology of
TNFr/OGP-like molecules and inhibitors thereof; stratification
of populations and generation of surrogate markers for
5   clinical trials; and the enhancement of TNFr/OGP-like related
small molecule drug discovery by aiding in the identification
of selective compounds in high throughput screens (HTS).

Selective Binding Agents

10   As used herein, the term "selective binding agent"
reffers to a molecule which has specificity for one or more
TNFr/OGP-like polypeptides. Suitable selective binding agents
include antibodies and dervatives thereof, polypeptides, and
samll molecules. Suitable selective binding agents may be   .
15 prepared using methods known in the art. An exemplary
TNFr/OGP-like polypeptide selective binding agent of the
present invention is capable of binding a certain portion of
the TNFr/OGP-like polypeptide thereby inhibiting the binding
of the polypeptide to the TNFr/OGP-like receptor(s).

20   Selective binding agents such as antibodies and antibody
fragemtns that bind TNFr/OGP-like are within the scope of the
present invention. The antibodies may be may be polyclonal
including monospecific polyclonal, monoclonal (mAbs),
recombinant, chimeric, humanized (such as CDR-grafted), human,
25 single chain, and/or bispecific, as well as fragements,
variants, or derivatives thereof. Antibody fragments include
those portions of the antibody which bind to an epitope on the
TNFr/OPG-like polypeptide. Examples of such fragments include
Fab and F(ab') fragments generated by enzymatic cleavage of
30 full-length antibodies. Other binding fragments include those
generated by recombinant DNA techniques, such as the
expression of recombinant plasmids containing nucleic acid
sequences encoding antibody variable regions.

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Polyclonal antibodies directed toward a TNFr/OPG-like
polypeptide generally are produced in animals (e.g., rabbits
or mice) by means of multiple subcutaneous or intraperitoneal
injections of TNFr/OPG-like polypeptide and an adjuvant. It
5   may be useful to conjugate a TNFr/OPG-like polypeptide, or a
variant, fragment, or derivative thereof to a carrier protein
that is immunogenic in the species to be immunized, such as
keyhole limpet heocyanin, serum, albumin, bovine
thyroglobulin, or soybean trypsin inhibitor. Also,
10   aggregating agents such as alum are used to enhance the immune
response. After immunization, the animals are bled and the
serum is assayed for anti-TNFr/OPG-like antibody titer.

Monoclonal antibodies directed toward TNFr/OPG-like
polypeptides are produced using any method which provides for
15   the production of antibody molecules by continuous cell lines
in culture. Examples of suitable methods for preparing
monoclonal antibodies include the hybridoma methods of Kohler
et al., Nature, 256:495-497 (1975) and the human B-cell
hybridoma method, Kozbor, J. Immunol., 133:3001 (1984);
20   Brodeur et al., Monoclonal Antibody Production Techniques and
Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987).
Also provided by the invention are hybridoma cell lines which
produce monoclonal antibodies reactive with TNFr/OPG-like
polypeptides.

25   Monoclonal antibodies of the invention may be modified
for use as therapeutics. One embodiment is a "chimeric"
antibody in which a portion of the heavy and/or light chain is
identical with or homologous to a corresponding sequence in
antibodies derived from a particular species or belonging to a
30   particular antibody class or subclass, while the remainder of
the chain(s) is identical with or homologous to a
corresponding sequence in antibodies derived from another
species or belonging to another antibody class or subclass.

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Also included are fragments of such antibodies, so long as
they exhibit the desired biological activity. See, U.S.
Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci.*,
81: 6851-6855 (1985).

5   In another embodiment, a monoclonal antibody of the
invention is a "humanized" antibody. Methods for humanizing
non-human antibodies are well known in the art. *See* U.S.
patent Nos. 5,585,089 and 5,693,762. Generally, a humanized
antibody has one or more amino acid residues introduced into
10   it from a source which is non-human. Humanization can be
performed, for example, using methods known in the art (Jones
et al., *Nature* 321: 522-525 (1986); Riechmann *et al.*, *Nature*,
332: 323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536
(1988)), by substituting at least a portion of a rodent
15   complementarity-determining region (CDR) for the corresponding
regions of a human antibody.

         Also encompassed by the invention are human antibodies
which bind TNFr/OPG-like polypeptides. Using transgentic
animals (e.g.) mice that are capable of producing a repertoire
20   of human antibodies in the absence of endogenous immunoglobin
production such antibodies are produced by immunization with a
TNFr/OPG-like antigen (*i.e.*, having at least 6 contiguous
amino acids), optionally conjugated to a carrier. *See* for
example, Jakobovits *et al.*, *Proc. Natl. Acad. Sci.*, 90: 2551-
25   2555 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993);
Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993). In one
method, such transgenic animals are produced by incapacitating
the endogenous loci encoding the heavy and light
immunoglobulin chains therein, and inserting loci encoding
30   human heavy and light chain proteins into the genome thereof.
Partially modified animals, that is those having less than the
full complement of modifications, are then cross-bred to
obtain an animal having all of the desired immune system

modifications.   When administered an immunogen, these
transgenic animals produce antibodies with human variable
regions, including human (rather than e.g., murine) amino acid
sequences, including variable regions which are immunospecific
5   for these antigens.   See PCT application nos. PCT/US96/05928
and PCT/US93/06926.   Additional methods are described in U.S.
Patent No. 5,545,807, PCT application nos. PCT/US91/245,
PCT/GB89/01207, and in EP 546073B1 and EP 546073A1.

Human antibodies may also be produced by the expression
10   of recombinant DNA in host cells or by expression in hybridoma
cells as described herein.

In an alternate emboidment, human antibdoies can be
produced from phage-display libraries (Hoogenboom et al., J.
Mol. Biol. 227: 381 (1991); Marks et al., J. Mol. Biol. 222:
15   581 (1991).   These processes mimic immune selection through
the display of antibody repertoires on the surface of
filamentous bacteriophage, and subsequent selection of phage
by their binding to an antigen of choice.   One such technique
is described in PCT Application no. PCT/US98/17364, which
20   describes the isolation of high affinity and functional
agonistic antibodies for MPL- and msk- receptors using such an
approach.

Chimeric, CDR grafted, and humanized antibodies are
typically produced by recombinant methods.   Nucleic acids
25   encoding the antibodies are introduced into host cells and
expressed using materials and procedures described herein.   In
a preferred embodiment, the antibodies are produced in
mammalian host cells, such as CHO cells.   Monoclonal (e.g.
human) antibodies may be produced by the expression of
30   recombinant DNA in host cells or by expression in hybridoma
cells as described herein.

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The anti-TNFr/OGP-like antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, Monoclonal Antibodies: A

5   Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of TNFr/OPG-like polypeptides. The antibodies will bind TNFr/OGP-like polypeptides with an affinity which is appropriate for the assay method being employed.

10 For diagnostic applications, in certain embodiments, anti-TNFr/OPG-like antibodies typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be

15 a radioisotope, such as ^3H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase. Bayer *et al.*, *Meth. Enz.*, 184: 138-163 (1990

20 Competitive binding assays rely on the ability of a labeled standard (*e.g.*, a TNFr/OPG-like polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (a TNFr/OPG-like polypeptide) for binding with a limited amount of anti TNFr/OPG-like antibody. The

25 amount of a TNFr/OPG-like polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the competition, so that the

30 standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two
antibodies, each capable of binding to a different immunogenic
portion, or epitope, of the protein to be detected and/or
quantitated. In a sandwich assay, the test sample analyte is
5   typically bound by a first antibody which is immobilized on a
solid support, and thereafter a second antibody binds to the
analyte, thus forming an insoluble three part complex. See,
e.g., U.S. Patent No. 4,376,110. The second antibody may
itself be labeled with a detectable moiety (direct sandwich
10 assays) or may be measured using an anti-immunoglobulin
antibody that is labeled with a detectable moiety (indirect
sandwich assays). For example, one type of sandwich assay is
an enzyme-linked immunosorbent assay (ELISA), in which case
the detectable moiety is an enzyme.

15   The selective binding agents of the invention, including
TNFr/OGP-like antibodies, may be used as therapeutics. These
therapeutic agents are generally agonists or antagonists, in
that they either enhance or reduce, respectively, at least one
of the biological activities of a TNFr/OPG-like polypeptide.
20 In one embodiment, antagonist antibodies of the invention are
antibodies or binding fragments thereof which are capable of
specifically binding to a TNFr/OPG-like polypeptide and which
are capable of inhibiting or eliminating the functional
activity of a TNFr/OPG-like polypeptide in vivo or in vitro.
25 In preferred embodiments, the selctive binidng agent e.g., an
antagonist antibody will inhibit the functional activity of a
TNFr/OPG-like polypeptide by at least about 50%, and
preferably by at least about 80%. In another embodiment, the
selective binding agent may be an antibody that is capable of
30 interacting with a TNFr/OPG-like binding partner (a ligand or
receptor) thereby inhibiting or eliminating TNFr/OPG-like
activity in vitro or in vivo. Selective binding agents,
including agonist and antagonist anti-TNFr/OPG-like antibodies

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are identified by screening assays which are well known in the art.

The anti-TNFr/OPG-like antibodies of the invention also are useful for *in vivo* imaging. An antibody labeled with a
5   detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. The antibody may be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other
10   detection means known in the art.

The invention also relates to a kit comprising TNFr/OPG-like selective binding agents (such as antibodies) and other reagents useful for detecting TNFr/OPG-like polypeptide levels in biological samples. Such reagents may include a secondary
15   activity, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

As noted, the TNFr/OPG-like receptor(s) recited herein are useful for identifying or developing novel agonists and antagonists of the TNFr/OPG-like signaling pathway. Such
20   agonists and antagonists include soluble TNFr/OPG-like receptor(s), anti-TNFr/OPG-like receptor antibodies, small molecules, or antisense oligonucleotides, and they may also be used for treating one or more of the diseases/disorders described herein.

25   **Additional Agonist and Antagonist Molecules**

As defined herein, agonist or antagonist molecules either enhance or reduce, respectively, at least one of the biological activities of a TNFr/OPG-like polypeptide. Antagonists are capable of interacting with the TNFr/OPG-like
30   receptor itself and/or with a TNFr/OPG-like binding partner (such as a ligand or receptor), thereby inhibiting or

eliminating TNFr/OPG-like polypeptide activity *in vitro* or *in vivo*. Agonists are those molecules that can specifically bind to the TNFr/OPG-like molecule and function like their native ligands to activate the receptor. Agonists can also interact

5   with a TNFr/OPG-like binding partner (such as a ligand) to enhance its binding to the TNFr/OPG-like polypeptides, thereby enhancing the biological activity of the TNFr/OPG-like molecule. It will be appreciated that the agonists and antagonists described herein are not limited to selective

10 binding agents. In addition to selective binding agents, other suitable agonist and antagonist molecules include, but are not limited to, soluble TNFr/OPG-like polypeptides, small molecules, and antisense oligonucleotides, any of which can be used for treating one or more disease or disorder, including

15 those described herein.

TNFr/OPG-like polypeptides can be used to clone TNFr/OPG-like ligand(s) using an "expression cloning" strategy. Radiolabeled (125-Iodine) TNFr/OPG-like polypeptide or "affinity/activity-tagged" TNFr/OPG-like polypeptide (such as

20 an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or a cell line or tissue that expresses TNFr/OPG-like ligand(s). RNA isolated from such cells or tissues can then be converted to cDNA, cloned into a mammalian expression vector, and transfected

25 into mammalian cells (for example, COS, or 293) to create an expression library. Radiolabeled or tagged TNFr/OPG-like polypeptide can then be used as an affinity reagent to identify and isolate the subset of cells in this library expressing TNFr/OPG-like ligand(s). DNA is then isolated from

30 these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing TNFr/OPG-like ligand(s) would be many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone

containing a TNFr/OPG-like ligand is isolated. Isolation of
TNFr/OPG-like ligand(s) is useful for identifying or
developing novel agonists and antagonists of the TNFr/OPG-like
signaling pathway. Such agonists and antagonists include
5   TNFr/OPG-like ligand(s), anti-TNFr/OPG-like ligand antibodies,
small molecules or antisense oligonucleotides.

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present
10   invention are non-human animals such as mice, rats, or other
rodents, rabbits, goats, or sheep, or other farm animals, in
which the gene (or genes) encoding a native TNFr/OPG-like
polypeptide has (have) been disrupted ("knocked out") such
that the level of expression of this gene or genes is (are)
15   significantly decreased or completely abolished. Such animals
may be prepared using techniques and methods such as those
described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals
such as mice, rats, or other rodents, rabbits, goats, or
20   sheep, or other farm animals, in which either the native form
of the TNFr/OPG-like polypeptide gene(s) for that animal or a
heterologous TNFr/OPG-like polypeptide gene(s) is (are) over
expressed by the animal, thereby creating a "transgenic"
animal. Such transgenic animals may be prepared using well
25   known methods such as those described in U.S. Patent No
5,489,743 and PCT application No. WO94/28122.

The present invention further includes non-human animals
in which the promoter for one or more of the TNFr/OPG-like
polypeptides of the present invention is either activated or
30   inactivated (e.g., by using homologous recombination methods)
to alter the level of expression of one or more of the native
TNFr/OPG-like polypeptides.

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These non-human animals may be used for drug candidate
screening. In such screening, the impact of a drug candidate
on the animal may be measured. For example, drug candidates
may decrease or increase the expression of the TNFr/OPG-like
5   polypeptide gene. In certain embodiments, the amount of
TNFr/OPG-like polypeptide, or a fragment(s), that is produced
may be measured after the exposure of the animal to the drug
candidate. Additionally, in certain embodiments, one may
detect the actual impact of the drug candidate on the animal.
10   For example, the overexpression of a particular gene may
result in, or be associated with, a disease or pathological
condition. In such cases, one may test a drug candidate's
ability to decrease expression of the gene or its ability to
prevent or inhibit a pathological condition. In other
15   examples, the production of a particular metabolic product
such as a fragment of a polypeptide, may result in, or be
associated with, a disease or pathological condition. In such
cases, one may test a drug candidate's ability to decrease the
production of such a metabolic product or its ability to
20   prevent or inhibit a pathological condition.

Assaying for Other Modulators of TNFr/OPG-Like Polypeptide Activity

In some situations, it may be desirable to identify
25   molecules that are modulators, *i.e.*, agonists or antagonists,
of the activity of TNFr/OPG-like polypeptide. Natural or
synthetic molecules that modulate TNFr/OPG-like polypeptides
may be identified using one or more screening assays, such as
those described herein. Such molecules may be administered
30   either in an *ex vivo* manner, or in an *in vivo* manner by
injection, or by oral delivery, implantation device, or the
like.

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"Test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to modulate (*i.e.*, increase or decrease) the activity of a TNFr/OPG-like polypeptide. Most commonly, a test molecule will interact directly with a

5   TNFr/OPG-like polypeptide. However, it is also contemplated that a test molecule may also modulate TNFr/OPG-like polypeptide activity indirectly, such as by affecting TNFr/OPG-like gene expression, or by binding to a TNFr/OPG-like binding partner (*e.g.*, receptor or ligand). In one

10   embodiment, a test molecule will bind to a TNFr/OPG-like polypeptide with an affinity constant of at least about 10^{-6} M, preferably about 10^{-8} M, more preferably about 10^{-9} M, and even more preferably about 10^{-10} M.

Methods for identifying compounds which interact with

15   TNFr/OPG-like polypeptides are encompassed by the present invention. In certain embodiments, a TNFr/OPG-like polypeptide is incubated with a test molecule under conditions which permit the interaction of the test molecule with a TNFr/OPG-like polypeptide, and the extent of the interaction

20   can be measured. The test molecule(s) can be screened in a substantially purified form or in a crude mixture. Test molecule(s) can be nucleic acid molecules, proteins, peptides, carbohydrates, lipids, or small molecular weight organic or inorganic compounds. Once a set of test molecules has been

25   identified as interacting with a TNFr/OPG-like polypeptide, the molecules may be further evaluated for their ability to increase or decrease TNFr/OPG-like polypeptide activity.

The measurement of the interaction of test molecules with TNFr/OPG-like polypeptides may be carried out in several

30   formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, test molecules are incubated with a TNFr/OPG-like polypeptide for a specified period of time, and TNFr/OPG-like polypeptide

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activity is determined by one or more assays described herein for measuring biological activity.

The interaction of test molecules with TNFr/OPG-like polypeptides may also be assayed directly using polyclonal or
5   monoclonal antibodies in an immunoassay. Alternatively, modified forms of TNFr/OPG-like polypeptides containing epitope tags as described herein may be used in solution and immunoassays.

In certain embodiments, a TNFr/OPG-like polypeptide
10   agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with TNFr/OPG-like polypeptide to regulate its activity. Potential protein antagonists of TNFr/OPG-like polypeptide include antibodies which interact with active regions of the
15   polypeptide and inhibit or eliminate at least one activity of TNFr/OPG-like molecules. Molecules which regulate TNFr/OPG-like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding a TNFr/OPG-like polypeptide, or are complementary to nucleic acids sequences
20   which direct or control the expression of TNFr/OPG-like polypeptide, and which act as anti-sense regulators of expression.

In the event that TNFr/OPG-like polypeptides display biological activity through an interaction with a binding
25   partner (e.g., a ligand), a variety of in vitro assays may be used to measure the binding of a TNFr/OPG-like polypeptide to the corresponding binding partner (such as a selective binding agent or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate
30   and/or the extent of binding of a TNFr/OPG-like polypeptide to its binding partner. In one assay, a TNFr/OPG-like polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled TNFr/OPG-like binding partner (for example,

iodinated TNFr/OPG-like binding partner) and the test
molecule(s) can then be added either one at a time (in either
order) or simultaneously to the wells. After incubation, the
wells can be washed and counted, using a scintillation

5   counter, for radioactivity to determine the extent to which
the binding partner bound to TNFr/OPG-like polypeptide.
Typically, the molecules will be tested over a range of
concentrations, and a series of control wells lacking one or
more elements of the test assays can be used for accuracy in

10   the evaluation of the results. An alternative to this method
involves reversing the "positions" of the polypeptides, *i.e.*,
immobilizing TNFr/OPG-like binding partner to the microtiter
plate wells, incubating with the test molecule and
radiolabeled TNFr/OPG-like polypeptide, and determining the

15   extent of TNFr/OPG-like polypeptide binding. See, for
example, chapter 18, *Current Protocols in Molecular Biology*,
Ausubel *et al.*, eds., John Wiley & Sons, New York, NY (1995).

As an alternative to radiolabelling, a TNFr/OPG-like
polypeptide or its binding partner may be conjugated to biotin

20   and the presence of biotinylated protein can then be detected
using streptavidin linked to an enzyme, such as horseradish
peroxidase (HRP) or alkaline phosphatase (AP), that can be
detected colorometrically, or by fluorescent tagging of
streptavidin. An antibody directed to a TNFr/OPG-like

25   polypeptide or to a TNFr/OPG-like binding partner and
conjugated to biotin may also be used and can be detected
after incubation with enzyme-linked streptavidin linked to AP
or HRP.

A TNFr/OPG-like polypeptide and a TNFr/OPG-like binding

30   partner can also be immobilized by attachment to agarose
beads, acrylic beads or other types of such inert solid phase
substrates. The substrate-protein complex can be placed in a
solution containing the complementary protein and the test

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compound. After incubation, the beads can be precipitated by
centrifugation, and the amount of binding between a TNFr/OPG-
like polypeptide and its binding partner can be assessed using
the methods described herein. Alternatively, the substrate-
5   protein complex can be immobilized in a column, and the test
molecule and complementary protein are passed through the
column. The formation of a complex between a TNFr/OPG-like
polypeptide and its binding partner can then be assessed using
any of the techniques set forth herein, *i.e.*, radiolabelling,
10   antibody binding, or the like.

Another *in vitro* assay that is useful for identifying a
test molecule which increases or decreases the formation of a
complex between a TNFr/OPG-like binding polypeptide and a
TNFr/OPG-like binding partner is a surface plasmon resonance
15   detector system such as the BIAcore assay system (Pharmacia,
Piscataway, NJ). The BIAcore system may be carried out using
the manufacturer's protocol. This assay essentially involves
the covalent binding of either TNFr/OPG-like polypeptide or a
TNFr/OPG-like binding partner to a dextran-coated sensor chip
20   which is located in a detector. The test compound and the
other complementary protein can then be injected, either
simultaneously or sequentially, into the chamber containing
the sensor chip. The amount of complementary protein that
binds can be assessed based on the change in molecular mass
25   which is physically associated with the dextran-coated side of
the sensor chip; the change in molecular mass can be measured
by the detector system.

In some cases, it may be desirable to evaluate two or
more test compounds together for their ability to increase or
30   decrease the formation of a complex between a TNFr/OPG-like
polypeptide and a TNFr/OPG-like binding partner. In these
cases, the assays set forth herein can be readily modified by
adding such additional test compound(s) either simultaneous

with, or subsequent to, the first test compound. The
remainder of the steps in the assay are as set forth herein.

        In vitro assays such as those described herein may be
used advantageously to screen large numbers of compounds for
5   effects on complex formation by TNFr/OPG-like polypeptide and
TNFr/OPG-like binding partner. The assays may be automated to
screen compounds generated in phage display, synthetic
peptide, and chemical synthesis libraries.

        Compounds which increase or decrease the formation of a
10   complex between a TNFr/OPG-like polypeptide and a TNFr/OPG-
like binding partner may also be screened in cell culture
using cells and cell lines expressing either TNFr/OPG-like
polypeptide or TNFr/OPG-like binding partner. Cells and cell
lines may be obtained from any mammal, but preferably will be
15   from human or other primate, canine, or rodent sources. The
binding of a TNFr/OPG-like polypeptide to cells expressing
TNFr/OPG-like binding partner at the surface is evaluated in
the presence or absence of test molecules, and the extent of
binding may be determined by, for example, flow cytometry
20   using a biotinylated antibody to a TNFr/OPG-like binding
partner. Cell culture assays can be used advantageously to
further evaluate compounds that score positive in protein
binding assays described herein.

        Cell cultures can also be used to screen the impact of a
25   drug candidate. For example, drug candidates may decrease or
increase the expression of the TNFr/OPG-like polypeptide gene.
In certain embodiments, the amount of TNFr/OPG-like
polypeptide or a fragment(s) that is produced may be measured
after exposure of the cell culture to the drug candidate. In
30   certain embodiments, one may detect the actual impact of the
drug candidate on the cell culture. For example, the
overexpression of a particular gene may have a particular
impact on the cell culture. In such cases, one may test a

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drug candidate's ability to increase or decrease the
expression of the gene or its ability to prevent or inhibit a
particular impact on the cell culture. In other examples, the
production of a particular metabolic product such as a
5   fragment of a polypeptide, may result in, or be associated
with, a disease or pathological condition. In such cases, one
may test a drug candidate's ability to decrease the production
of such a metabolic product in a cell culture.

A yeast two-hybrid system (Chien et al., Proc. Natl.
10   Acad. Sci. USA, 88:9578-9583, 1991) can be used to identify
novel polypeptides that bind to, or interact with, TNFr/OPG-
like polypeptides. As an example, a yeast-two hybrid bait
construct can be generated in a vector (such as the pAS2-1
from Clontech) which encodes a yeast GAL4-DNA binding domain
15   fused to the TNFr/OPG-like polynucleotide. This bait
construct may be used to screen human cDNA libraries wherein
the cDNA library sequences are fused to GAL4 activation
domains. Positive interactions will result in the activation
of a reporter gene such as -Gal. Positive clones emerging
20   from the screening may be characterized further to identify
interacting proteins.

P38 Inhibitors

A new approach to intervention between the extracellular
stimulus and the secretion of IL-1 and TNF from the cell
25   involves blocking signal transduction through inhibition of a
kinase which lies on the signal pathway. One example is
through inhibition of P-38 (also called "RK" or "SAPK-2", Lee
et al., Nature, 372:739 (1994)), a known ser/thr kinase (clone
reported in Han et al., Biochimica Biophysica Acta, 1265:224-
30   227 (1995)). A linear relationship has been shown for
effectiveness in a competitive binding assay to P-38, and the
same inhibitor diminishing the levels of IL-1 secretion from

monocytes following LPS stimulation. Following LPS
stimulation of monocytes, the levels of messenger RNA for TNF-
a have been shown to increase 100 fold, but the protein levels
of TNF-a are increased 10,000 fold. Thus, a considerable

5   amplification of the TNF signaling occurs at the translational
level. Following LPS stimulation of monocytes in the presence
of a P-38 inhibitor, the levels of mRNA are not affected, but
the levels of final TNF protein are dramatically reduced (up
to 80-90% depending on the effectiveness of the P-38

10   inhibitor). Thus, the above experiments lend strong support
to the conclusion that inhibition of P-38 leads to diminished
translational efficiency. Further evidence that TNF is under
translational control is found in the deletion experiments of
Beutler et al. and Lee, wherein segments of 3' untranslated

15   mRNA (3' UTR) are removed resulting in high translational
efficiency for TNF. More importantly, the P-38 inhibitors did
not have an effect on the level of TNF (i.e., translational
efficiency) when the appropriate segments of TNF mRNA are
deleted. Thus, the correlative data between the level of

20   binding of inhibitors to P-38 and the diminished IL-1 and TNF
levels following LPS stimulation with the same inhibitors,
plus the above biochemical evidence regarding the effect of P-
38 inhibitors on translational efficiency of both TNF and IL-
1 make a strong cause and effect relationship. The role of P-

25   38 in the cell is still being delineated; so therefore, other
beneficial effects regarding inflammatory diseases or other
disease states obtained from its inhibition maybe forthcoming.

Elevated levels of TNF and/or IL-1 may contribute to the
onset, etiology, or exacerbate a number of disease states,

30   including, but not limited to: rheumatoid arthritis;
osteoarthritis; rheumatoid spondylitis; gouty arthritis;
inflammatory bowel disease; adult respiratory distress
syndrome (ARDS); psoriasis; Crohn's disease; allergic
rhinitis; ulcerative colitis; anaphylaxis; contact dermatitis;

asthma; antiviral therapy including those viruses sensitive to
TNF inhibition - HIV-1, HIV-2, HIV-3, cytomegalovirus (CMV),
influenza, adenovirus, and the herpes viruses including HSV-1,
HSV-2, and herpes zoster; muscle degeneration; cachexia;
5   Reiter's syndrome; type II diabetes; bone resorption diseases;
graft vs. host reaction; ischemia reperfusion injury;
atherosclerosis; brain trauma; Alzheimer's disease; multiple
sclerosis; cerebral malaria; sepsis; septic shock; toxic shock
syndrome; fever and mylagias due to infection.

10   Substituted imidazole, pyrrole, pyridine, pyrimidine and
the like compounds have been described for use in the
treatment of cytokine mediated diseases by inhibition of
proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF.
Substituted imidazoles for use in the treatment of cytokine
15   mediated diseases have been described in U.S. Patent No.
5,593,992; WO 93/14081; WO 97/18626; WO 96/21452; WO 96/21654;
WO 96/40143; WO 97/05878; WO 97/05878; (each of which is
incorporated herein by reference in its entirety).
Substituted imidazoles for use in the treatment of
20   inflammation has been described in US Pat. 3,929,807 (which is
incorporated herein by reference in its entirety).
Substituted pyrrole compounds for use in the treatment of
cytokine mediated diseases have been described in WO 97/05877;
WO 97/05878; WO 97/16426; WO 97/16441; and WO 97/16442 (each
25   of which is incorporated herein by reference in its entirety).
Substituted aryl and heteroaryl fused pyrrole compounds for
use in the treatment of cytokine mediated diseases have been
described in WO 98/22457 (which is incorporated herein by
reference in its entirety). Substituted pyridine, pyrimidine,
30   pyrimidinone and pyridazine compounds for use in the treatment
of cytokine mediated diseases have been described in WO
98/24780; WO 98/24782; WO 99/24404; and WO 99/32448 (each of
which is incorporated herein by reference in its entirety).

Internalizing Proteins

     The tat protein sequence (from HIV) can be used to
internalize proteins into a cell. See e.g., Falwell et al.,
Proc. Natl. Acad. Sci.USA, 91:664-668 (1994). For example, an
5   11 amino acid sequence (YGRKKRRQRRR: SEQ ID NO: 21) of the HIV
tat protein (termed the "protein"protein transduction
domain",domain", or TAT PDT) has been described as mediating
delivery across the cytoplasmic membrane and the nuclear
membrane of a cell. See Schwarze et al., Science,285:1569-
10   285:1569-1572 (1999); and Nagahara et al., Nature Medicine,
4:1449-1452 (1998). In these procedures, FITC-constructs
(FITC-GGGGYGRKKRRQRRR; SEQ ID NO: 22) are prepared which bind
to cells as observed by fluorescence-fluorescence-activated
cell sorting (FACS) analysis, and these constructs penetrate
15   tissues after i.p. adminstration. Next, tat-bgal fusion
proteins are constructed. Cells treated with this construct
demonstrated b-gal activity. Following injection, a number of
tissues, including liver, kidney, lung, heart, and brain
tissue, have been found to demonstrate expression using these
20   procedures. It is believed that these constructions underwent
some degree of unfolding in order to enter the cell; as such,
refolding may be required after entering the cell.

     It will thus be appreciated that the tat protein sequence
may be used to internalize a desired protein or polypeptide
25   into a cell. For example, using the tat protein sequence, an
TNFr/OPG-like antagonist (such as an anti-TNFr/OPG-like
selective binding agent, small molecule, soluble receptor, or
antisense oligonucleotide) can be administered intracellularly
to inhibit the activity of an TNFr/OPG-like molecule. As used
30   herein, the term "TNFr/OPG-like molecule" refers to both
TNFr/OPG-like nucleic acid molecules and TNFr/OPG-like
polypeptides as defined herein. Where desired, the TNFr/OPG-
like protein itself may also be internally administered to a

cell using these procedures. See also, Strauss, E.,
"Introducing Proteins Into the Body's Cells", Science,
285:1466-1467 (1999).

Cell Source Identification Using TNFr/OPG-Like Polypeptides

5   In accordance with certain embodiments of the invention,
it may be useful to be able to determine the source of a
certain cell type associated with a TNFr/OPG-like polypeptide.
For example, it may be useful to determine the origin of a
disease or pathological condition as an aid in selecting an
10   appropriate therapy. In other embodiments, one may use the
TNFr/OPG-like polypeptide to make antibodies that are specific
for TNFr/OPG-like polypeptide.

Therapeutic Uses

         Bone tissue provides support for the body and consists of
15   mineral (largely calcium and phosphorous), a matrix of
collagenous and noncollagenous proteins, and cells. Three
types of cells found in bone, osteocytes, osteoblasts, and
osteoclasts, are involved in the dynamic process by which bone
is continually formed and resorbed. Osteoblasts promote
20   formation of bone tissue whereas osteoclasts are associated
with resorption. Resorption, or the dissolution of bone matrix
and mineral, is a fast and efficient process compared to bone
formation and can release large amounts of mineral from bone.
Osteoclasts are involved in the regulation of the normal
25   remodeling of skeletal tissue and in resorption induced by
hormones. For instance, resorption is stimulated by the
secretion of parathyroid hormone in response to decreasing
concentrations of calcium ion in extracellular fluids. In
contrast, inhibition of resorption is the principal function
30   of calcitonin. In addition, metabolites of vitamin D alter
the responsiveness of bone to parathyroid hormone and
calcitonin.

Following skeletal maturity, the amount of bone in the skeleton reflects the balance (or imbalance) of bone formation and bone resorption. Peak bone mass occurs after skeletal maturity prior to the fourth decade. Between the fourth and

5   fifth decades, the equilibrium shifts and bone resorption dominates. The inevitable decrease in bone mass with advancing years starts earlier in females than males and is distinctly accelerated after menopause in some females (principally those of Caucasian and Asian descent).

10   Osteopenia is a condition relating generally to any decrease in bone mass to below normal levels. Such a condition may arise from a decrease in the rate of bone synthesis or an increase in the rate of bone destruction or both. The most common form of osteopenia is primary

15   osteoporosis, also referred to as postmenopausal and senile osteoporosis. This form of osteoporosis is a consequence of the universal loss of bone with age and is usually a result of increase in bone resorption with a normal rate of bone formation. About 25 to 30 percent of all white females in the

20   United States develop symptomatic osteoporosis. A direct relationship exists between osteoporosis and the incidence of hip, femoral, neck, and inter-trochanteric fracture in women 45 years and older. Elderly males develop symptomatic osteoporosis between the ages of 50 and 70, but the disease

25   primarily affects females.

The cause of postmenopausal and senile osteoporosis is unknown. Several factors have been identified which may contribute to the condition. They include alteration in hormone levels accompanying aging, and inadequate calcium

30   consumption attributed to decreased intestinal absorption of calcium and other minerals. Treatments have usually included hormone therapy or dietary supplements in an attempt to retard the process. To date, however, an effective treatment for bone loss does not exist.

The invention provides for a method of treating,
preventing, or diagnosing the diseases and disorders recited
herein using a therapeutically effective amount of TNFr/OPG-
like polypeptide. By way of example, the disease or disorder
5   may be any disease or disorder characterized by a net bone
loss (such as osteopenia or osteolysis). TNFr/OPG-like
polypeptides may be used for example to suppress the rate of
bone resorption. Thus, treatment may be done to reduce the
rate of bone resorption where the resorption rate is above
10   normal, or to reduce bone resorption to below normal levels in
order to compensate for below normal levels of bone formation.

Conditions which are treatable with TNFr/OPG-like
polypeptides include the following:

- Osteoporosis, such as primary osteoporosis, endocrine
15   osteoporosis (hyperthyroidism, hyperparathryoidism,
Cushing's syndrome, and acromegaly), hereditary and
congenital forms of osteoporosis (osteogenesis
imperfecta, homocystinuria, Menkes' syndrome, and
Riley-Day syndrome), and osteoporosis due to
20   immobilization of extremities.

- Paget's disease of bone (osteitis deformans) in adults
and juveniles;

- Osteomyelitis, or an infectious lesion in bone, leading
to bone loss;

25   - Hypercalcemia resulting from solid tumors (breast,
lung, and kidney) and hematologic malignacies (multiple
myeloma, lymphoma, and leukemia), idiopathic
hypercalcemia, and hypercalcemia associated with
hyperthryoidism and renal function disorders;

30   - Osteopenia following surgery, induced by steroid
administration, and associated with disorders of the

small and large intestine and with chronic hepatic and
renal diseases;

5

- Osteonecrosis, or bone cell death, associated with
  traumatic injury or nontraumatic necrosis associated
  with Gaucher's disease, sickle cell anemia, systemic
  lupus erythematosus, rheumatoid arthritis, periodontal
  disease, osteolytic metastasis, and other conditions.

Other diseases associated with undesirable levels of one
or more of TNF, OPG, IL-1, and/or the present TNFr/OPG-like

10   polypeptide itself are encompassed within the scope of the
invention. Undesirable levels include excessive and/or sub-
normal levels of TNF, OPG, IL-1, and/or the present TNFr/OPG-
like polypeptides described herein.

It is understood that the TNFr/OPG-like polypeptides may

15   be used alone or in conjunction with other factors for the
treatment of bone disorders. In one embodiment, TNFr/OPG-like
polypeptides are used in conjunction with a therapeutically
effective amount of one or more agents which stimulate bone
formation or decrease bone destruction. Such agents include,

20   but are not limited to, the bone morphogenic factors (BMP's)
designated BMP-1 through BMP-12; transforming growth factor-β
(TGF-β) and TGF-β family members; interleukin-1 (IL-1)
inhibitors; TNF-α inhibitors; parathyroid hormone and analogs
thereof, parathyroid related protein and analogs thereof; E

25   series prostaglandins; bisphosphonates (such as alendronate
and others); bone-enhancing minerals such as fluoride and
calcium; non-steroidal anti-inflammatory drugs (NSAIDs),
including COX-2 inhibitors, such as Celebrex™ and Vioxx™;
immunosuppressants, such as methotrexate or leflunomide;

30   serine protease inhibitors such as secretory leukocyte
protease inhibitor (SLPI); IL-6 inhibitors (e.g., antibodies
to IL-6), IL-8 inhibitors (e.g., antibodies to IL-8); IL-18

inhibitors (*e.g.*, IL-18 binding protein or IL-18 antibodies);
interleukin-1 converting enzyme (ICE) modulators; fibroblast
growth factors FGF-1 to FGF-10 and FGF modulators; PAF
antagonists; keratinocyte growth factor (KGF), KGF-related
5   molecules, or KGF modulators; matrix metalloproteinase (MMP)
modulators; Nitric oxide synthase (NOS) modulators, including
modulators of inducible NOS; modulators of glucocorticoid
receptor; modulators of glutamate receptor; modulators of
lipopolysaccharide (LPS) levels; and noradrenaline and
10   modulators and mimetics thereof.

A non-exclusive list of acute and chronic diseases
treatable in accordance with the invention include, but is not
limited to, the following: cachexia/anorexia; cancer (*e.g.*,
leukemias); chronic fatigue syndrome; coronary conditions and
15   indications, including congestive heart failure, coronary
restenosis, myocardial infarction, and coronary artery bypass
graft; depression; diabetes (*e.g.*, juvenile onset Type 1 and
diabetes mellitus); endometriosis, endometritis, and related
conditions; fibromyalgia or analgesia; graft versus host
20   rejection; hyperalgesia; inflammatory bowel diseases,
including Crohn's disease and *Clostridium difficile*-associated
diarrhea; ischemic, including cerebral ischemia (brain injury
as a result of trauma, epilepsy, hemorrhage or stroke, each of
which may lead to neurodegeneration); lung diseases (*e.g.*,
25   adult respiratory distress syndrome, asthma, and pulmonary
fibrosis); multiple sclerosis; neuroinflammatory diseases;
ocular diseases and conditions, including corneal transplant,
ocular degeneration and uveitis; pain, including cancer-
related pain; pancreatitis; periodontal diseases; prostatitis
30   (bacterial or non-bacterial) and related conditions; psoriasis
and related conditions; pulmonary fibrosis; reperfusion
injury; rheumatic diseases (*e.g.*, rheumatoid arthritis,
osteoarthritis, juvenile (rheumatoid) arthritis, seronegative
polyarthritis, ankylosing spondylitis, Reiter's syndrome and

reactive arthritis, Still's disease, psoriatic arthritis, enteropathic arthritis, polymyositis, dermatomyositis, scleroderma, systemic sclerosis, vasculitis (e.g., Kawasaki's disease), cerebral vasculitis, Lyme disease, staphylococcal-

5   induced ("septic") arthritis, Sjögren's syndrome, rheumatic fever, polychondritis and polymyalgia rheumatica and giant cell arteritis); septic shock; side effects from radiation therapy; systemic lupus erythematosus; temporal mandibular joint disease; thyroiditis; tissue transplantation or an

10   inflammatory condition resulting from strain, sprain, cartilage damage, trauma, orthopedic surgery, infection (e.g., HIV, Clostridium difficile and related species) or other disease process.

     As contemplated by the present invention, a TNFr/OPG-like

15   polypeptide may be administered as an adjunct to other therapy and also with other pharmaceutical agents suitable for the indication being treated. A TNFr/OPG-like polypeptide and any of one or more additional therapies or pharmaceutical agents may be administered separately, sequentially, or

20   simultaneously.

     In a specific embodiment, the present invention is directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more interleukin-1 inhibitors

25   for the treatment of TNF-responsive disease. Classes of interleukin-1 inhibitors include interleukin-1 receptor antagonists (any compound capable of specifically preventing activation of cellular receptors to IL-1) such as IL-1ra, as described herein; anti-IL-1 receptor monoclonal antibodies

30   (e.g., EP 623674, the disclosure of which is hereby incorporated by reference); IL-1 binding proteins such as soluble IL-1 receptors (e.g., U.S. Patent Nos. 5,492,888, 5,488,032, 5,464,937, 5,319,071 and 5,180,812, the disclosures of which are hereby incorporated by reference); anti-IL-1

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monoclonal antibodies (e.g., WO 95/01997, WO 94/02627, WO
90/06371, U.S. Patent No. 4,935,343, EP 364778, EP 267611 and
EP 220063, the disclosures of which are hereby incorporated by
reference); IL-1 receptor accessory proteins (e.g.,
5   WO 96/23067), and other compounds and proteins which block in
vivo synthesis or extracellular release of IL-1.

        Interleukin-1 receptor antagonist (IL-1ra) is a human
protein that acts as a natural inhibitor of interleukin-1.
Interleukin-1 receptor antagonists, as well as the methods of
10   making and methods of using thereof, are described in U.S.
Patent No. 5,075,222; WO 91/08285; WO 91/17184; AU 9173636; WO
92/16221; WO 93/21946; WO 94/06457; WO 94/21275; FR 2706772;
WO 94/21235; DE 4219626; WO 94/20517; WO 96/22793 and WO
97/28828, the disclosures of which are incorporated herein by
15   reference. The proteins include glycosylated as well as non-
glycosylated IL-1 receptor antagonists.

        Specifically, three exemplary forms of IL-1ra (IL-1raα,
IL-1raβ and IL-1rax), are disclosed and described in U.S.
Patent No. 5,075,222. Methods for producing IL-1 inhibitors,
20   particularly IL-1ras, are also disclosed in the 5,075,222
patent.

        An additional class of interleukin-1 inhibitors includes
compounds capable of specifically preventing activation of
cellular receptors to IL-1. Such compounds include IL-1
25   binding proteins, such as soluble receptors and monoclonal
antibodies. Such compounds also include monoclonal antibodies
to the receptors.

        A further class of interleukin-1 inhibitors includes
compounds and proteins which block in vivo synthesis and/or
30   extracellular release of IL-1. Such compounds include agents
which affect transcription of IL-1 genes or processing of IL-1
preproteins.

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In a specific embodiment, the present invention is directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with secreted or soluble human fas antigen or

5   recombinant versions thereof (WO 96/20206 and Mountz et al., *J. Immunology*, 155:4829-4837; and EP 510 691). WO 96/20206 discloses secreted human fas antigen (native and recombinant, including an Ig fusion protein), methods for isolating the genes responsible for coding the soluble recombinant human fas

10   antigen, methods for cloning the gene in suitable vectors and cell types, and methods for expressing the gene to produce the inhibitors. EP 510 691 teaches DNAs coding for human fas antigen, including soluble fas antigen, vectors expressing for said DNAs and transformants transfected with the vector. When

15   administered parenterally, doses of a secreted or soluble fas antigen fusion protein each are generally from about 1 microgram/kg to about 100 micrograms/kg.

Present treatment of diseases associated with TNF, including acute and chronic inflammation such as rheumatic

20   diseases, commonly involves the use of first line drugs for control of pain and inflammation; these drugs are classified as non-steroidal, anti-inflammatory drugs (NSAIDs). Secondary treatments include corticosteroids, slow acting antirheumatic drugs (SAARDs) or disease modifying (DM) drugs. Information

25   regarding the following compounds can be found in The Merck Manual of Diagnosis and Therapy, Sixteenth Edition, Merck, Sharp & Dohme Research Laboratories, Merck & Co., Rahway, NJ (1992) and in Pharmaprojects, PJB Publications Ltd.

In a specific embodiment, the present invention is

30   directed to the use of a TNFr/OPG-like polypeptide and any of one or more NSAIDs for the treatment of the diseases and disorder recited herein, including acute and chronic inflammation such as rheumatic diseases; and graft versus host disease. NSAIDs owe their anti-inflammatory action, at least

in part, to the inhibition of prostaglandin synthesis (Goodman
and Gilman in "The Pharmacological Basis of Therapeutics,"
MacMillan 7th Edition (1985)). NSAIDs can be characterized
into at least nine groups: (1) salicylic acid derivatives;
5   (2) propionic acid derivatives; (3) acetic acid derivatives;
(4) fenamic acid derivatives; (5) carboxylic acid derivatives;
(6) butyric acid derivatives; (7) oxicams; (8) pyrazoles and
(9) pyrazolones.

       In another embodiment, the present invention is directed
10   to the use of a TNFr/OPG-like polypeptide in combination
(pretreatment, post-treatment, or concurrent treatment) with
any of one or more salicylic acid derivatives, prodrug esters
or pharmaceutically acceptable salts thereof. Such salicylic
acid derivatives, prodrug esters and pharmaceutically
15   acceptable salts thereof comprise: acetaminosalol, aloxiprin,
aspirin, benorylate, bromosaligenin, calcium acetylsalicylate,
choline magnesium trisalicylate, magnesium salicylate, choline
salicylate, diflusinal, etersalate, fendosal, gentisic acid,
glycol salicylate, imidazole salicylate, lysine
20   acetylsalicylate, mesalamine, morpholine salicylate, 1-
naphthyl salicylate, olsalazine, parsalmide, phenyl
acetylsalicylate, phenyl salicylate, salacetamide,
salicylamide O-acetic acid, salsalate, sodium_salicylate and
sulfasalazine. Structurally related salicylic acid
25   derivatives having similar analgesic and anti-inflammatory
properties are also intended to be encompassed by this group.

       In an additional specific embodiment, the present
invention is directed to the use of a TNFr/OPG-like
polypeptide in combination (pretreatment, post-treatment or
30   concurrent treatment) with any of one or more propionic acid
derivatives, prodrug esters or pharmaceutically acceptable
salts thereof. The propionic acid derivatives, prodrug esters
and pharmaceutically acceptable salts thereof comprise:
alminoprofen, benoxaprofen, bucloxic acid, carprofen,

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dexindoprofen, fenoprofen, flunoxaprofen, fluprofen,
flurbiprofen, furcloprofen, ibuprofen, ibuprofen aluminum,
ibuproxam, indoprofen, isoprofen, ketoprofen, loxoprofen,
miroprofen, naproxen, naproxen sodium, oxaprozin,
5   piketoprofen, pimeprofen, pirprofen, pranoprofen, protizinic
acid, pyridoxiprofen, suprofen, tiaprofenic acid and
tioxaprofen. Structurally related propionic acid derivatives
having similar analgesic and anti-inflammatory properties are
also intended to be encompassed by this group.

10   In another specific embodiment, the present invention is
directed to the use of a TNFr/OPG-like polypeptide in
combination (pretreatment, post-treatment or concurrent
treatment) with any of one or more acetic acid derivatives,
prodrug esters or pharmaceutically acceptable salts thereof.
15   The acetic acid derivatives, prodrug esters and
pharmaceutically acceptable salts thereof comprise:
acemetacin, alclofenac, amfenac, bufexamac, cinmetacin,
clopirac, delmetacin, diclofenac potassium, diclofenac sodium,
etodolac, felbinac, fenclofenac, fenclorac, fenclozic acid,
20   fentiazac, furofenac, glucametacin, ibufenac, indomethacin,
isofezolac, isoxepac, lonazolac, metiazinic acid, oxametacin,
oxpinac, pimetacin, proglumetacin, sulindac, talmetacin,
tiaramide, tiopinac, tolmetin, tolmetin sodium, zidometacin
and zomepirac. Structurally related acetic acid derivatives
25   having similar analgesic and anti-inflammatory properties are
also intended to be encompassed by this group.

In yet another more specific embodiment, the present
invention is directed to the use of a TNFr/OPG-like
polypeptide in combination (pretreatment, post-treatment or
30   concurrent treatment) with any of one or more fenamic acid
derivatives, prodrug esters or pharmaceutically acceptable
salts thereof. The fenamic acid derivatives, prodrug esters
and pharmaceutically acceptable salts thereof comprise:
enfenamic acid, etofenamate, flufenamic acid, isonixin,

meclofenamic acid, meclofenamate sodium, medofenamic acid, mefenamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid and ufenamate. Structurally related fenamic acid derivatives having similar analgesic and anti-

5   inflammatory properties are also intended to be encompassed by this group.

In still another more specific embodiment, the present invention is directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment or

10   concurrent treatment) with any of one or more carboxylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The carboxylic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof which can be used comprise: clidanac, diflunisal, flufenisal, inoridine,

15   ketorolac and tinoridine. Structurally related carboxylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is

20   directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more butyric acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The butyric acid derivatives, prodrug esters and

25   pharmaceutically acceptable salts thereof comprise: bumadizon, butibufen, fenbufen and xenbucin. Structurally related butyric acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

30   In a further specific embodiment, the present invention is directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more oxicams, prodrug esters or pharmaceutically acceptable salts thereof. The oxicams,

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prodrug esters and pharmaceutically acceptable salts thereof
comprise: droxicam, enolicam, isoxicam, piroxicam, sudoxicam,
tenoxicam and 4-hydroxyl-1,2-benzothiazine 1,1-dioxide 4-(N-
phenyl)-carboxamide. Structurally related oxicams having
5 ·similar analgesic and anti-inflammatory properties are also
intended to be encompassed by this group.

In still another specific embodiment, the present
invention is directed to the use of a TNFr/OPG-like
polypeptide in combination (pretreatment, post-treatment, or
10 concurrent treatment) with any of one or more pyrazoles,
prodrug esters or pharmaceutically acceptable salts thereof.
The pyrazoles, prodrug esters and pharmaceutically acceptable
salts thereof which may be used comprise: difenamizole and
epirizole. Structurally related pyrazoles having similar
15 analgesic and anti-inflammatory properties are also intended
to be encompassed by this group.

In an additional specific embodiment, the present
invention is directed to the use of a TNFr/OPG-like
polypeptide in combination (pretreatment, post-treatment or
20 concurrent treatment) with any of one or more pyrazolones,
prodrug esters or pharmaceutically acceptable salts thereof.
The pyrazolones, prodrug esters and pharmaceutically
acceptable salts thereof which may be used comprise: apazone,
azapropazone, benzpiperylon, feprazone, mofebutazone,
25 morazone, oxyphenbutazone, phenylbutazone, pipebuzone,
propylphenazone, ramifenazone, suxibuzone and
thiazolinobutazone. Structurally related pyrazalones having
similar analgesic and anti-inflammatory properties are also
intended to be encompassed by this group.

30   In another specific embodiment, the present invention is
directed to the use of a TNFr/OPG-like polypeptide in
combination (pretreatment, post-treatment or concurrent
treatment) with any of one or more of the following NSAIDs:

ε-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-
hydroxybutyric acid, amixetrine, anitrazafen, antrafenine,
bendazac, bendazac lysinate, benzydamine, beprozin,
broperamole, bucolome, bufezolac, ciproquazone, cloximate,

5   dazidamine, deboxamet, detomidine, difenpiramide,
difenpyramide, difisalamine, ditazol, emorfazone, fanetizole
mesylate, fenflumizole, floctafenine, flumizole, flunixin,
fluproquazone, fopirtoline, fosfosal, guaimesal, guaiazolene,
isonixirn, lefetamine HCl, leflunomide, lofemizole,

10   lotifazole, lysin clonixinate, meseclazone, nabumetone,
nictindole, nimesulide, orgotein, orpanoxin, oxaceprol,
oxapadol, paranyline, perisoxal, perisoxal citrate, pifoxime,
piproxen, pirazolac, pirfenidone, proquazone, proxazole,
thielavin B, tiflamizole, timegadine, tolectin, tolpadol,

15   tryptamid and those designated by company code number such as
480156S, AA861, AD1590, AFP802, AFP860, AI77B, AP504, AU8001,
BPPC, BW540C, CHINOIN 127, CN100, EB382, EL508, F1044, FK-506,
GV3658, ITF182, KCNTEI6090, KME4, LA2851, MR714, MR897, MY309,
ONO3144, PR823, PV102, PV108, R830, RS2131, SCR152, SH440,

20   SIR133, SPAS510, SQ27239, ST281, SY6001, TA60, TAI-901 (4-
benzoyl-1-indancarboxylic acid), TVX2706, U60257, UR2301 and
WY41770. Structurally related NSAIDs having similar analgesic
and anti-inflammatory properties to the NSAIDs are also
intended to be encompassed by this group.

25   In still another specific embodiment, the present
invention is directed to the use of a TNFr/OPG-like
polypeptide in combination (pretreatment, post-treatment, or
concurrent treatment) with any of one or more corticosteroids,
prodrug esters or pharmaceutically acceptable salts thereof

30   for the treatment of TNF-responsive diseases, including acute
and chronic inflammation such as rheumatic diseases, graft
versus host disease and multiple sclerosis. Corticosteroids,
prodrug esters and pharmaceutically acceptable salts thereof
include hydrocortisone and compounds which are derived from

hydrocortisone, such as 21-acetoxypregnenolone, alclomerasone, algestone, amcinonide, beclomethasone, betamethasone, betamethasone valerate, budesonide, chloroprednisone, clobetasol, clobetasol propionate, clobetasone, clobetasone
5   butyrate, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacon, desonide, desoximerasone, dexamethasone, diflorasone, diflucortolone, difluprednate, enoxolone, fluazacort, flucloronide, flumethasone, flumethasone pivalate, flucinolone acetonide, flunisolide,
10 fluocinonide, fluorocinolone acetonide, fluocortin butyl, fluocortolone, fluocortolone hexanoate, diflucortolone valerate, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandenolide, formocortal, halcinonide, halometasone, halopredone acetate,
15 hydrocortamate, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone phosphate, hydrocortisone 21-sodium succinate, hydrocortisone tebutate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate,
20 prednisolone, prednisolone 21-diedryaminoacetate, prednisolone sodium phosphate, prednisolone sodium succinate, prednisolone sodium 21-m-sulfobenzoate, prednisolone sodium 21-stearoglycolate, prednisolone tebutate, prednisolone 21-trimethylacetate, prednisone, prednival, prednylidene,
25 prednylidene 21-diethylaminoacetate, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide and triamcinolone hexacetonide. Structurally related corticosteroids having similar analgesic and anti-inflammatory properties are also intended to be encompassed by
30 this group.

In another specific embodiment, the present invention is directed to the use of a TNFr/OPG-like polypeptide in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more slow-acting antirheumatic

drugs (SAARDs) or disease modifying antirheumatic drugs
(DMARDS), prodrug esters or pharmaceutically acceptable salts
thereof for the treatment of TNF-responsive diseases,
including acute and chronic inflammation such as rheumatic
5   diseases, graft versus host disease and multiple sclerosis.
SAARDs or DMARDS, prodrug esters and pharmaceutically
acceptable salts thereof comprise: allocupreide sodium,
auranofin, aurothioglucose, aurothioglycanide, azathioprine,
brequinar sodium, bucillamine, calcium 3-aurothio-2-propanol-
10   1-sulfonate, chlorambucil, chloroquine, clobuzarit,
cuproxoline, cyclophosphamide, cyclosporin, dapsone, 15-
deoxyspergualin, diacerein, glucosamine, gold salts (e.g.,
cycloquine gold salt, gold sodium thiomalate, gold sodium
thiosulfate), hydroxychloroquine, hydroxychloroquine sulfate,
15   hydroxyurea, kebuzone, levamisole, lobenzarit, melittin, 6-
mercaptopurine, methotrexate, mizoribine, mycophenolate
mofetil, myoral, nitrogen mustard, D-penicillamine, pyridinol
imidazoles such as SKNF86002 and SB203580, rapamycin, thiols,
thymopoietin and vincristine. Structurally related SAARDs or
20   DMARDs having similar analgesic and anti-inflammatory
properties are also intended to be encompassed by this group.

        In a further specific embodiment, the present invention
is directed to the use of a TNFr/OPG-like polypeptide in
combination (pretreatment, post-treatment, or concurrent
25   treatment) with any of one or more COX2 inhibitors, prodrug
esters or pharmaceutically acceptable salts thereof for the
treatment of TNF-responsive diseases, including acute and
chronic inflammation. Examples of COX2 inhibitors, prodrug
esters or pharmaceutically acceptable salts thereof include,
30   for example, celecoxib. Structurally related COX2 inhibitors
having similar analgesic and anti-inflammatory properties are
also intended to be encompassed by this group.

        In yet another specific embodiment, the present invention
is directed to the use of a TNFr/OPG-like polypeptide in

combination (pretreatment, post-treatment or concurrent
treatment) with any of one or more antimicrobials, prodrug
esters or pharmaceutically acceptable salts thereof for the
treatment of TNF-responsive diseases, including acute and
5   chronic inflammation. Antimicrobials include, for example,
the broad classes of penicillins, cephalosporins and other
beta-lactams, aminoglycosides, azoles, quinolones, macrolides,
rifamycins, tetracyclines, sulfonamides, lincosamides and
polymyxins. The penicillins include, but are not limited to
10   penicillin G, penicillin V, methicillin, nafcillin, oxacillin,
cloxacillin, dicloxacillin, floxacillin, ampicillin,
ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanate,
hetacillin, cyclacillin, bacampicillin, carbenicillin,
carbenicillin indanyl, ticarcillin, ticarcillin/clavulanate,
15   azlocillin, mezlocillin, peperacillin, and mecillinam. The
cephalosporins and other beta-lactams include, but are not
limited to cephalothin, cephapirin, cephalexin, cephradine,
cefazolin, cefadroxil, cefaclor, cefamandole, cefotetan,
cefoxitin, ceruroxime, cefonicid, ceforadine, cefixime,
20   cefotaxime, moxalactam, ceftizoxime, cetriaxone,
cephoperazone, ceftazidime, imipenem and aztreonam. The
aminoglycosides include, but are not limited to streptomycin,
gentamicin, tobramycin, amikacin, netilmicin, kanamycin and
neomycin. The azoles include, but are not limited to
25   fluconazole. The quinolones include, but are not limited to
nalidixic acid, norfloxacin, enoxacin, ciprofloxacin,
ofloxacin, sparfloxacin and temafloxacin. The macrolides
include, but are not limited to erythomycin, spiramycin and
azithromycin. The rifamycins include, but are not limited to
30   rifampin. The tetracyclines include, but are not limited to
spicycline, chlortetracycline, clomocycline, demeclocycline,
deoxycycline, guamecycline, lymecycline, meclocycline,
methacycline, minocycline, oxytetracycline, penimepicycline,
pipacycline, rolitetracycline, sancycline, senociclin and

tetracycline. The sulfonamides include, but are not limited to sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, sulfisoxazole and co-trimoxazole (trimethoprim/sulfamethoxazole). The lincosamides include,
5   but are not limited to clindamycin and lincomycin. The polymyxins (polypeptides) include, but are not limited to polymyxin B and colistin.

In certain preferred embodiments, a polypeptide comprising TNFr/OPG-like polypeptides is used in conjunction
10   with particular therapeutic molecules to treat various inflammatory conditions, autoimmune conditions, and other conditions leading to bone loss. Depending on the condition and the desired level of treatment, two, three, or more agents may be administered, separately, simultaneously, or
15   sequentially. These agents may be provided together by inclusion in the same formulation or inclusion in a treatment kit, or they may be provided separately. When administered by gene therapy, the genes encoding the protein agents may be included in the same vector, optionally under the control of
20   the same promoter region, or in separate vectors. Particularly preferred molecules in the aforementioned classes are as follows.

   •   IL-1 inhibitors: IL-1ra proteins, and soluble IL-1 receptors. The most preferred IL-1 inhibitor is anakinra.

25   •   TNF-α inhibitors: soluble tumor necrosis factor receptor type I (sTNF-RI; -RI is also called the p55 receptor); soluble tumor necrosis factor receptor type II (also called the p75 receptor); and monoclonal antibodies that bind the TNF receptor. Most preferred is sTNF-RI as described
30   in WO 98/24463, etanercept (Enbrel[®]), and Avakine[®]. Exemplary TNF-α inhibitors are described in EP 422 339, EP 308 378, EP 393 438, EP 398 327, and EP 418 014.

   •   Serine protease inhibitors: Such as SLPI. These

inhibitors also may be viewed as exemplary LPS modulators, as SLPI has been shown to inhibit LPS responses. Jin *et al.* (1997), *Cell*, 88(3):417-26.

Particularly preferred methods of treatment concern use of TNF-α inhibitors and IL-1 inhibitors in conjunction with polypeptides comprising TNFr/OPG-like polypeptides. Such polypeptides may be used with either or both TNF-α inhibitors and IL-1 inhibitors for treatment of conditions such as rheumatoid arthritis and multiple sclerosis.

It will be appreciated that other diseases associated with undesirable levels of one or more of TNF, OPG, and/or the present TNFr/OPG-like polypeptides themselves are encompassed within the scope of the invention. Undesirable levels include excessive and/or sub-normal levels of TNF, OPG, and/or the TNFr/OPG-like polypeptides described herein.

TNF-α inhibitors may act by downregulating or inhibiting TNF production, binding free TNF, interfering with TNF binding to its receptor, or interfering with modulation of TNF signaling after binding to its receptor. The term "TNF-α inhibitor" thus includes solubilized TNF receptors, antibodies to TNF, antibodies to TNF receptor, inhibitors of TNF-α converting enzyme (TACE), and other molecules that affect TNF activity.

TNF-α inhibitors of various kinds are disclosed in the art, including the following references:

European patent applications 308 378; 422 339; 393 438; 398 327; 412 486; 418 014, 417 563, 433 900; 464 533; 512 528; 526 905; 568 928; 663 210; 542 795; 818 439; 664 128; 542 795; 741 707; 874 819 ; 882 714; 880 970; 648 783; 731 791; 895 988; 550 376; 882 714; 853 083; 550 376; 943 616;

U.S. Patent Nos. 5,136,021; 5,929,117; 5,948,638; 5,807,862; 5,695,953; 5,834,435; 5,817,822; 5,830,742;

5,834,435; 5,851,556; 5,853,977; 5,359,037; 5,512,544;

5,695,953; 5,811,261; 5,633,145; 5,863,926; 5,866,616;

5,641,673; 5,869,677; 5,869,511; 5,872,146; 5,854,003;

5,856,161; 5,877,222; 5,877,200; 5,877,151; 5,886,010;

5   5,869,660; 5,859,207; 5,891,883; 5,877,180; 5,955,480;

5,955,476; 5,955,435;

International (WO) patent applications 90/13575,
91/03553, 92/01002, 92/13095, 92/16221, 93/07863, 93/21946,
93/19777, 95/34326, 96/28546, 98/27298, 98/30541, 96/38150,

10   96/38150, 97/18207, 97/15561, 97/12902, 96/25861, 96/12735,
96/11209, 98/39326, 98/39316, 98/38859, 98/39315, 98/42659,
98/39329, 98/43959, 98/45268, 98/47863, 96/33172, 96/20926,
97/37974, 97/37973, 96/35711, 98/51665, 98/43946, 95/04045,
98/56377, 97/12244, 99/00364, 99/00363, 98/57936, 99/01449,

15   99/01139, 98/56788, 98/56756, 98/53842, 98/52948, 98/52937,
99/02510, 97/43250, 99/06410, 99/06042, 99/09022, 99/08688,
99/07679, 99/09965, 99/07704, 99/06041, 99/37818, 99/37625,
97/11668;

Japanese (JP) patent applications 10147531, 10231285,

20   10259140, and 10130149, 10316570, 11001481, and 127,800/1991;
German (DE) application 19731521; British (GB) applications 2
218 101, 2 326 881, 2 246 569.

For purposes of this invention, the molecules disclosed
in these references including the sTNFRs and variants and

25   derivatives of the sTNFRs disclosed in the references, (see
below) are collectively termed "TNF-α inhibitors."

For example, EP 393 438 and EP 422 339 teach the amino
acid and nucleic acid sequences of a soluble TNF receptor type
I (also known as sTNFR-I or 30kDa TNF inhibitor) and a soluble

30   TNF receptor type II (also known as sTNFR-II or 40kDa TNF
inhibitor), collectively termed "sTNFRs", including modified
forms thereof (e.g., fragments, functional derivatives and
variants). EP 393 438 and EP 422 339 also disclose methods

for isolating the genes responsible for coding the inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors.

sTNFR-I and sTNFR-II are members of the nerve growth
5   factor/TNF receptor superfamily of receptors which includes the nerve growth factor receptor (NGF), the B cell antigen CD40, 4-1BB, the rat T-cell antigen MRC OX40, the fas antigen, and the CD27 and CD30 antigens (Smith et al. (1990), *Science*, *248*:1019-1023). The most conserved feature among this group
10   of cell surface receptors is the cysteine-rich extracellular ligand binding domain, which can be divided into four repeating motifs of about forty amino acids and which contains 4-6 cysteine residues at positions which are well conserved (Smith et al. (1990), *supra*).

15   EP 393 438 teaches a 40kDa TNF inhibitor Δ51 and a 40kDa TNF inhibitor Δ53. These are truncated versions of the full-length recombinant 40kDa TNF inhibitor protein wherein 51 or 53 amino acid residues, respectively, at the carboxyl terminus of the mature protein are removed.

20   PCT Application No. PCT/US97/12244 teaches truncated forms of sTNFR-I and sTNFR-II which do not contain the fourth domain (amino acid residues Thr^{127}-Asn^{161} of sTNFR-I and amino acid residues Pro^{141}-Thr^{179} of sTNFR-II); a portion of the third domain (amino acid residues Asn^{111}-Cys^{126} of sTNFR-I and
25   amino acid residues Pro^{123}-Lys^{140} of sTNFR-II); and, optionally, which do not contain a portion of the first domain (amino acid residues Asp^{1}-Cys^{19} of sTNFR-I and amino acid residues Leu^{1}-Cys^{32} of sTNFR-II).

IL-1 inhibitors include any protein capable of
30   specifically preventing activation of cellular receptors to IL-1, which may result from any number of mechanisms. Such mechanisms include downregulating IL-1 production, binding

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free IL-1, interfering with IL-1 binding to its receptor, interfering with formation of the IL-1 receptor complex (*i.e.*, association of IL-1 receptor with IL-1 receptor accessory protein), or interfering with modulation of IL-1 signaling after binding to its receptor. Classes of interleukin-1 inhibitors include:

- Interleukin-1 receptor antagonists such as IL-1ra, as described herein;

- Anti-IL-1 receptor monoclonal antibodies (*e.g.*, EP 623674);

- IL-1 binding proteins such as soluble IL-1 receptors (*e.g.*, U.S. Pat. No. 5,492,888, U.S. Pat. No. 5,488,032, and U.S. Pat. No. 5,464,937, U.S. Pat. No. 5,319,071, and U.S. Pat. No. 5,180,812;

- Anti-IL-1 monoclonal antibodies (*e.g.*, WO 9501997, WO 9402627, WO 9006371, U.S. Pat. No. 4,935,343, EP 364778, EP 267611 and EP 220063;

- IL-1 receptor accessory proteins and antibodies thereto (*e.g.*, WO 96/23067);

- Inhibitors of interleukin-1β converting enzyme (ICE) or caspase I, which can be used to inhibit IL-1 beta production and secretion;

- Interleukin-1β protease inhibitors;

- Other compounds and proteins which block *in vivo* synthesis or extracellular release of IL-1.

Exemplary IL-1 inhibitors are disclosed in the following references:

US Pat. Nos. 5747444; 5359032; 5608035; 5843905; 5359032; 5866576; 5869660; 5869315; 5872095; 5955480;

International (WO) patent applications 98/21957,

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96/09323, 91/17184, 96/40907, 98/32733, 98/42325, 98/44940,
98/47892, 98/56377, 99/03837, 99/06426, 99/06042, 91/17249,
98/32733, 98/17661, 97/08174, 95/34326, 99/36426, and
99/36415;

5   European (EP) patent applications 534978 and 894795; and
French patent application FR 2762514.

TNFr/OPG-Like Compositions and Administration

         Therapeutic compositions are within the scope of the
present invention. Such compositions may comprise a
10   therapeutically effective amount of a TNFr/OPG-like
polypeptide, including a fragment, variant, derivative, or one
or more selective binding agents in admixture with a
pharmaceutically acceptable agent such as a pharmaceutically
acceptable formulation agent.

15   TNFr/OPG-like molecule pharmaceutical compositions
typically include a therapeutically or prophylactically
effective amount of TNFr/OPG-like polypeptide, nucleic acid
molecule, or selective binding agent in admixture with one or
more pharmaceutically and physiologically acceptable
20   formulation agents selected for suitability with the mode of
administration. Suitable formulation materials or
pharmaceutically acceptable agents include, but are not
limited to, antioxidants, preservatives, coloring, flavoring
and diluting agents, emulsifying agents, suspending agents,
25   solvents, fillers, bulking agents, buffers, delivery vehicles,
diluents, excipients and/or pharmaceutical adjuvants. For
example, a suitable vehicle or carrier may be water for
injection, physiological saline solution, or artificial
cerebrospinal fluid, possibly supplemented with other
30   materials common in compositions for parenteral
administration. Neutral buffered saline or saline mixed with
serum albumin are further exemplary vehicles.

The term "pharmaceutically acceptable carrier" or
"physiologically acceptable carrier" as used herein refers to
one or more formulation materials suitable for accomplishing
or enhancing the delivery of the TNFr/OPG-like polypeptide,
5   nucleic acid molecule or selective binding agent as a
pharmaceutical composition.

Acceptable formulation materials preferably are nontoxic
to recipients and are preferably inert at the dosages and
concentrations employed. The materials may include buffers
10   such as phosphate, citrate, or other organic acids;
antioxidants such as ascorbic acid; low molecular weight
polypeptides; proteins, such as serum albumin, gelatin, or
immunoglobulins; hydrophilic polymers such as
polyvinylpyrrolidone; amino acids (such as glycine, glutamine,
15   asparagine, arginine or lysine); monosaccharides,
disaccharides, and other carbohydrates including glucose,
mannose, or dextrins; chelating agents such as ethylenediamine
tetraacetic acid (EDTA); sugar alcohols such as mannitol or
sorbitol; salt-forming counterions such as sodium; and/or
20   nonionic surfactants such as tween, pluronics, or polyethylene
glycol (PEG).

Typically, a TNFr/OPG-like molecule pharmaceutical
composition will be administered in the form of a composition
comprising a purified polypeptide, in conjunction with one or
25   more physiologically acceptable agents. It will be
appreciated that when used herein, the term "TNFr/OPG-like
molecule pharmaceutical composition" also encompasses
compositions containing a nucleic acid molecule or selective
binding agent of the present invention.

30   Neutral buffered saline or saline mixed with serum
albumin are exemplary appropriate carriers. Other standard
pharmaceutically acceptable agents such as diluents and
excipients may be included as desired. For example, the

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TNFr/OPG-like polypeptide product may be formulated as a
lyophilizate using appropriate excipients such as sucrose.
Other exemplary pharmaceutical compositions comprise Tris
buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-
5   5.5, which may further include sorbitol or a suitable
substitute therefor.

        The primary vehicle or carrier in a pharmaceutical
composition may be either aqueous or non-aqueous in nature.
For example, a suitable vehicle or carrier may be water for
10   injection, physiological saline solution, solution or
artificial cerebrospinal fluid, possibly supplemented with
other materials common in compositions for parenteral
administration. Neutral buffered saline or saline mixed with
serum albumin are further exemplary vehicles. Other exemplary
15   pharmaceutical compositions comprise Tris buffer of about pH
7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may
further include sorbitol or a suitable substitute therefor.
In one embodiment of the present invention, TNFr/OPG-like
polypeptide compositions may be prepared for storage by mixing
20   the selected composition having the desired degree of purity
with optional formulation agents (Remington's Pharmaceutical
Sciences, supra) in the form of a lyophilized cake or an
aqueous solution. Further, the TNFr/OPG-like polypeptide
product may be formulated as a lyophilizate using appropriate
25   excipients such as sucrose.

        In addition, the composition may contain other
formulation materials for modifying, maintainingor preserving,
for example, the pH, osmolarity, viscosity, clarity, color,
sterility, stability, rate of dissolution or release,
30   adsorption or pentration of the composition, or odor of the
formulation. Similarly, the composition may contain
additional formulation materials for modifying or maintaining
the rate of release of TNFr/OPG-like polypeptide, nucleic acid
molecule or selective binding agent, or for promoting the

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absorption or penetration of such TNFr/OPG-like molecules.

The TNFr/OPG-like molecule pharmaceutical compositions can be administered parenterally. Alternatively, the compositions may be administered through the digestive tract, such as orally, or by inhalation. When parenterally administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such pharmaceutically acceptable compositions, with due regard to pH, isotonicity, stability and the like, is within the skill of the art.

A particularly suitable vehicle for parenteral injection is sterile distilled water in which a TNFr/OPG-like polypeptide is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

The pharmaceutical compositions of the present invention may include other components, for example parenterally acceptable preservatives, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite) and surfactants, as are well known in the art. For example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol, and the like. Suitable preservatives include, but are not limited to, benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, and the like. Hydrogen peroxide may also be used as preservative. Suitable

cosolvents are for example glycerin, propylene glycol and
polyethylene glycol. Suitable complexing agents are for
example caffeine, polyvinylpyrrolidone, beta-cyclodextrin or
hydroxypropyl-beta-cyclodextrin. Suitable surfactants or
5   wetting agents include sorbitan esters, polysorbates such as
polysorbate 80, tromethamine, lecithin, cholesterol,
tyloxapal, and the like. The buffers can be conventional
buffers such as borate, citrate, phosphate, bicarbonate, or
Tris-HCl.

10   The formulation components are present in concentrations
that are acceptable to the site of administration. For
example, buffers are used to maintain the composition at
physiological pH or at slightly lower pH, typically within a
pH range of from about 5 to about 8.

15   In one embodiment of the present invention, TNFr/OPG-like
polypeptide compositions may be prepared for storage by mixing
the selected composition having the desired degree of purity
with optional physiologically acceptable carriers, excipients,
or stabilizers (Remington's Pharmaceutical Sciences, 18th
20   Edition, A.R. Gennaro, ed., Mack Publishing Company [1990]) in
the form of a lyophilized cake or an aqueous solution. The
optimal pharmaceutical formulation will be determined by one
skilled in the art depending upon, for example, the intended
route of administration, delivery format, and desired dosage.
25   See for example, Remington's Pharmaceutical Sciences, pp.
1435-1712. Such compositions may influence the physical
state, stability, rate of in vivo release, and rate of in vivo
clearance of the present TNFr/OPG-like polypeptides.
An effective amount of a TNFr/OPG-like polypeptide
30   composition to be employed therapeutically will depend, for
example, upon the therapeutic objectives such as the
indication for which the TNFr/OPG-like polypeptide is being
used, the route of administration, and the condition of the
patient. Accordingly, the clinician may titer the dosage and

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modify the route of administration to obtain the optimal
therapeutic effect. A typical dosage may range from about 0.1
µg/kg to up to about 100 mg/kg or more, depending on the
factors mentioned above. In other embodiments, the dosage may
5   range from 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to
about 100 mg/kg; or 0.1 µg/kg up to about 100 mg/kg; or 1
µg/kg up to about 100 mg/kg.

        The frequency of dosing will depend upon the
pharmokinetic parameters of the TNFr/OPG-like molecule in the
10   formulation used. Typically, a clinician will administer the
composition until a dosage is reached that achieves the
desired effect. The composition may therefore be administered
as a single dose, or as two or more doses (which may or may
not contain the same amount of the desired molecule) over
15   time, or as a continuous infusion via implantation device or
catheter.

        One skilled in the art will appreciate that the
appropriate dosage levels for treatment will thus vary
depending, in part, upon the molecule delivered, the
20   therapeutic context, type of disorder under treatment, the
age, and general health of the recipient.

        The TNFr/OPG-like molecule pharmaceutical composition to
be used for in vivo administration typically must be sterile.
This may be accomplished by filtration through sterile
25   filtration membranes. Where the composition is lyophilized,
sterilization using these methods may be conducted either
prior to, or following, lyophilization and reconstitution.
The composition for parenteral administration may be stored in
lyophilized form or in solution. In addition, parenteral
30   compositions generally are placed into a container having a
sterile access port, for example, an intravenous solution bag
or vial having a stopper pierceable by a hypodermic injection
needle.

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Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-

5   use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having

10   a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

Pharmaceutical compositions such as (1) slow-release

15   formulations, (2) inhalant mists, or (3) orally active formulations are also envisioned. The TNFr/OPG-like molecule pharmaceutical composition generally is formulated for parenteral administration. Such parenterally administered therapeutic compositions are typically in the form of a

20   pyrogen-free, parenterally acceptable aqueous solution comprising the desired TNFr/OPG-like molecule in a pharmaceutically acceptable vehicle. The TNFr/OPG-like molecule pharmaceutical compositions also may include particulate preparations of polymeric compounds such as

25   polylactic acid, polyglycolic acid, etc. or the introduction of the molecule into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation.

In one embodiment, a pharmaceutical composition may be

30   formulated for inhalation. For example, TNFr/OPG-like polypeptide may be formulated as a dry powder for inhalation. TNFr/OPG-like polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery, with or without a liquefied propellant. In yet

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another embodiment, solutions may be nebulized. Pulmonary
administration is further described in PCT application no.
PCT/US94/001875, which describes pulmonary delivery of
chemically modified proteins.

5   It is also contemplated that certain formulations may be
administered through the digestive tract, such as orally. In
one embodiment of the present invention, TNFr/OPG-like
polypeptides which are administered in this fashion can be
formulated with or without those carriers customarily used in
10   the compounding of solid dosage forms such as tablets and
capsules. For example, a capsule may be designed to release
the active portion of the formulation at the point in the
gastrointestinal tract when bioavailability is maximized and
pre-systemic degradation is minimized. Additional agents can
15   be included to facilitate absorption of the TNFr/OPG-like
polypeptide. Diluents, flavorings, low melting point waxes,
vegetable oils, lubricants, suspending agents, tablet
disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an
20   effective quantity of TNFr/OPG-like molecules in a mixture
with non-toxic excipients which are suitable for the
manufacture of tablets. By dissolving the tablets in sterile
water, or other appropriate vehicle, solutions can be prepared
in unit dose form. Suitable excipients include, but are not
25   limited to, inert diluents, such as calcium carbonate, sodium
carbonate or bicarbonate, lactose, or calcium phosphate; or
binding agents, such as starch, gelatin, or acacia; or
lubricating agents such as magnesium stearate, stearic acid,
or talc.

30   Additional TNFr/OPG-like pharmaceutical compositions will
be evident to those skilled in the art, including formulations
involving TNFr/OPG-like molecules in combination with one or
more other therapeutic agents and TNFr/OPG-like polypeptide in
sustained release or controlled-delivery formulations.

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Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example,
5   PCT/US93/00829 which describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions.

Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped
10   articles, *e.g.* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, $\underline{22}$:547-556 [1983]), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J.*
15   *Biomed. Mater. Res.*, $\underline{15}$:167-277 [1981] and Langer, *Chem. Tech.*, $\underline{12}$:98-105 [1982]), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the
20   art. See *e.g.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, $\underline{82}$:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

Regardless of the manner of administration, the specific dose may be calculated according to body weight, body surface area or organ size. Further refinement of the appropriate
25   dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical
30   composition is in accord with known methods, *e.g.* oral, inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intraocular,

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intraarterial, intraportal, or intralesional routes, or by
sustained release systems or implantation device. Where
desired, the compositions may be administered continuously by
infusion, by bolus injection or by implantation device.

5   Alternatively or additionally, the composition may be
administered locally via implantation into the affected area
of a membrane, sponge, or other appropriate material on to
which the desired molecule has been absorbed or encapsulated.
Where an implantation device is used, the device may be
10   implanted into any suitable tissue or organ, and delivery of
the desired molecule may be directly through the device via
diffusion, time release bolus, or via continuous
administration, or via catheter using continuous infusion.

It will further be appreciated that the TNFr/OPG-like
15   polypeptides, including fragments, variants, and derivatives,
may be employed alone, together, or in combination with other
polypeptides and pharmaceutical compositions. For example,
the TNFr/OPG-like polypeptides may be used in combination with
cytokines, growth factors, antibiotics, anti-inflammatories,
20   and/or chemotherapeutic agents as is appropriate for the
indication being treated.

In some cases, it may be desirable to use TNFr/OPG-like
pharmaceutical compositions in an *ex vivo* manner. In such
instances, cells, tissues, or organs that have been removed
25   from the patient are exposed to TNFr/OPG-like pharmaceutical
compositions after which the cells, tissues and/or organs are
subsequently implanted back into the patient.

In other cases, a TNFr/OPG-like polypeptide can be
delivered by implanting certain cells that have been
30   genetically engineered, using methods such as those described
herein, to express and secrete the polypeptides. Such cells
may be animal or human cells, and may be autologous,

heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation

5   materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

10   Additional embodiments of the present invention relate to cells and methods (*e.g.*, homologous recombination and/or other recombinant production methods) for both the *in vitro* production of therapeutic polypeptides by means of homologous recombination and for the production and delivery of

15   therapeutic polypeptides by gene therapy or cell therapy.

It is further envisioned that TNFr/OPG-like polypeptides can be produced *in vitro* or *in vivo* by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already

20   containing DNA encoding TNFr/OPG-like polypeptides. For example, homologous recombination methods may be used to modify a cell that contains a normally transcriptionally silent TNFr/OPG-like gene, or an under expressed gene, and thereby produce a cell which expresses therapeutically

25   efficacious amounts of TNFr/OPG-like polypeptides. Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes. Kucherlapati, *Prog. in Nucl. Acid Res. & Mol. Biol.*, 36:301, 1989. The basic technique was

30   developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas *et al.*, *Cell*, 44:419-428, 1986; Thomas and Capecchi, *Cell*, 51:503-512, 1987; Doetschman *et al.*, *Proc. Natl. Acad. Sci.*, 85:8583-8587, 1988)

or to correct specific mutations within defective genes
(Doetschman *et al.*, *Nature*, 330:576-578, 1987). Exemplary
homologous recombination techniques are described in U.S.
Patent No. 5,272,071 (EP 9193051, EP Publication No. 505500;
5   PCT/US90/07642, International Publication No. WO 91/09955).

Through homologous recombination, the DNA sequence to be
inserted into the genome can be directed to a specific region
of the gene of interest by attaching it to targeting DNA. The
targeting DNA is a nucleotide sequence that is complementary
10   (homologous) to a region of the genomic DNA. Small pieces of
targeting DNA that are complementary to a specific region of
the genome are put in contact with the parental strand during
the DNA replication process. It is a general property of DNA
that has been inserted into a cell to hybridize, and
15   therefore, recombine with other pieces of endogenous DNA
through shared homologous regions. If this complementary
strand is attached to an oligonucleotide that contains a
mutation or a different sequence or an additional nucleotide,
it too is incorporated into the newly synthesized strand as a
20   result of the recombination. As a result of the proofreading
function, it is possible for the new sequence of DNA to serve
as the template. Thus, the transferred DNA is incorporated
into the genome.

Attached to these pieces of targeting DNA are regions of
25   DNA which may interact with or control the expression of a
TNFr/OPG-like polypeptide, *e.g.*, flanking sequences. For
example, a promoter/enhancer element, a suppresser, or an
exogenous transcription modulatory element is inserted in the
genome of the intended host cell in proximity and orientation
30   sufficient to influence the transcription of DNA encoding the
desired TNFr/OPG-like polypeptide. The control element
controls a portion of the DNA present in the host cell genome.
Thus, the expression of TNFr/OPG-like polypeptide may be

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achieved not by transfection of DNA that encodes the TNFr/OPG-like gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide
5   the endogenous gene sequence with recognizable signals for transcription of a TNFr/OPG-like polypeptide.

In an exemplary method, the expression of a desired targeted gene in a cell (i.e., a desired endogenous cellular gene) is altered via homologous recombination into the
10   cellular genome at a preselected site by the introduction, of DNA which includes at least a regulatory sequence, an exon and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit
15   (in which the regulatory sequence, the exon and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

20   Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at physiologically significant levels in the cell as obtained.
25   The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regualtion or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

30   One method by which homologous recombination can be used to increase, or cause, TNFr/OPG-like polypeptide production from a cell's endogenous TNFr/OPG-like gene involves first using homologous recombination to place a recombination

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sequence from a site-specific recombination system (*e.g.*,
Cre/loxP, FLP/FRT) (Sauer, *Current Opinion In Biotechnology*,
5:521-527, 1994; Sauer, *Methods In Enzymology*, 225:890-900,
1993) upstream (that is, 5' to) of the cell's endogenous

5   genomic TNFr/OPG-like polypeptide coding region. A plasmid
containing a recombination site homologous to the site that
was placed just upstream of the genomic TNFr/OPG-like
polypeptide coding region is introduced into the modified cell
line along with the appropriate recombinase enzyme. This

10   recombinase causes the plasmid to integrate, via the plasmid's
recombination site, into the recombination site located just
upstream of the genomic TNFr/OPG-like polypeptide coding
region in the cell line (Baubonis and Sauer, *Nucleic Acids
Res.*, 21:2025-2029, 1993; O'Gorman *et al.*, *Science*, 251:1351-

15   1355, 1991). Any flanking sequences known to increase
transcription (*e.g.*, enhancer/promoter, intron, translational
enhancer), if properly positioned in this plasmid, would
integrate in such a manner as to create a new or modified
transcriptional unit resulting in *de novo* or increased

20   TNFr/OPG-like polypeptide production from the cell's
endogenous TNFr/OPG-like gene.

A further method to use the cell line in which the site
specific recombination sequence had been placed just upstream
of the cell's endogenous genomic TNFr/OPG-like polypeptide

25   coding region is to use homologous recombination to introduce
a second recombination site elsewhere in the cell line's
genome. The appropriate recombinase enzyme is then introduced
into the two-recombination-site cell line, causing a
recombination event (deletion, inversion, translocation)

30   (Sauer, *Current Opinion In Biotechnology*, 5:521-527, 1994;
Sauer, *Methods In Enzymology*, 225:890-900, 1993) that would
create a new or modified transcriptional unit resulting in *de
novo* or increased TNFr/OPG-like polypeptide production from
the cell's endogenous TNFr/OPG-like gene.

An additional approach for increasing, or causing, the expression of TNFr/OPG-like polypeptide from a cell's endogenous TNFr/OPG-like gene involves increasing, or causing, the expression of a gene or genes (*e.g.*, transcription
5 factors) and/or decreasing the expression of a gene or genes (*e.g.*, transcriptional repressors) in a manner which results in *de novo* or increased TNFr/OPG-like polypeptide production from the cell's endogenous TNFr/OPG-like gene. This method includes the introduction of a non-naturally occurring
10 polypeptide (*e.g.*, a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased TNFr/OPG-like polypeptide production from the cell's endogenous TNFr/OPG-like gene results.

15   The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site.
20 The targeting sequence in the DNA construct directs the integration of elements (a)-(d) into a target gene in a cell such that the elements (b)-(d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more
25 targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that the elements of (b)-(f) are operatively linked to the endogenous gene. The
30 targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

If the sequence of a particular gene is known, such as
the nucleic acid sequence encoding a TNFr/OPG-like polypeptide
presented herein, a piece of DNA that is complementary to a
selected region of the gene can be synthesized or otherwise

5   obtained, such as by appropriate restriction of the native DNA
at specific recognition sites bounding the region of interest.
This piece serves as a targeting sequence upon insertion into
the cell and will hybridize to its homologous region within
the genome. If this hybridization occurs during DNA

10 replication, this piece of DNA, and any additional sequence
attached thereto, will act as an Okazaki fragment and will be
incorporated into the newly synthesized daughter strand of
DNA. The present invention, therefore, includes nucleotides
encoding a TNFr/OPG-like polypeptide, which nucleotides may be

15 used as targeting sequences.

TNFr/OPG-like polypeptide cell therapy, e.g., the
implantation of cells producing TNFr/OPG-like polypeptides, is
also contemplated. This embodiment involves implanting cells
capable of synthesizing and secreting a biologically active

20 form of TNFr/OPG-like polypeptide. Such TNFr/OPG-like
polypeptide-producing cells can be cells that are natural
producers of TNFr/OPG-like polypeptides or may be recombinant
cells whose ability to produce TNFr/OPG-like polypeptides has
been augmented by transformation with a gene encoding the

25 desired TNFr/OPG-like polypeptide or with a gene augmenting
the expression of TNFr/OPG-like polypeptide. Such a
modification may be accomplished by means of a vector suitable
for delivering the gene as well as promoting its expression
and secretion. In order to minimize a potential immunological

30 reaction in patients being administered a TNFr/OPG-like
polypeptide, as may occur with the administration of a
polypeptide of a foreign species, it is preferred that the
natural cells producing TNFr/OPG-like polypeptide be of human
origin and produce human TNFr/OPG-like polypeptide. Likewise,

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it is preferred that the recombinant cells producing TNFr/OPG-
like polypeptide be transformed with an expression vector
containing a gene encoding a human TNFr/OPG-like polypeptide.

Implanted cells may be encapsulated to avoid the
5   infiltration of surrounding tissue. Human or non-human animal
cells may be implanted in patients in biocompatible,
semipermeable polymeric enclosures or membranes that allow the
release of TNFr/OPG-like polypeptide, but that prevent the
destruction of the cells by the patient's immune system or by
10 other detrimental factors from the surrounding tissue.
Alternatively, the patient's own cells, transformed to produce
TNFr/OPG-like polypeptides *ex vivo*, may be implanted directly
into the patient without such encapsulation.

Techniques for the encapsulation of living cells are
15 known in the art, and the preparation of the encapsulated
cells and their implantation in patients may be routinely
accomplished. For example, Baetge *et al.* (WO95/05452;
PCT/US94/09299) describe membrane capsules containing
genetically engineered cells for the effective delivery of
20 biologically active molecules. The capsules are biocompatible
and are easily retrievable. The capsules encapsulate cells
transfected with recombinant DNA molecules comprising DNA
sequences coding for biologically active molecules operatively
linked to promoters that are not subject to down regulation *in*
25 *vivo* upon implantation into a mammalian host. The devices
provide for the delivery of the molecules from living cells to
specific sites within a recipient. In addition, see U.S.
Patent Nos. 4,892,538, 5,011,472, and 5,106,627. A system for
encapsulating living cells is described in PCT Application
30 WO91/10425 of Aebischer *et al.* See also, PCT Application
WO91/10470 of Aebischer *et al.*, Winn *et al.*, *Exper. Neurol.*,
113:322-329 (1991), Aebischer *et al.*, *Exper. Neurol.*, 111:269-
275 (1991); and Tresco *et al.*, ASAIO, 38:17-23 (1992).

In vivo and *in vitro* gene therapy delivery of TNFr/OPG-
like polypeptides is also envisioned. *In vivo* gene therapy
may be accomplished by introducing the gene encoding TNFr/OPG-
like polypeptide into cells via local injection of a TNFr/OPG-
5   like nucleic acid molecule or by other appropriate viral or
non-viral delivery vectors. Hefti, *Neurobiology*, 25:1418-1435
(1994). For example, a nucleic acid molecule encoding a
TNFr/OPG-like polypeptide may be contained in an adeno-
associated virus vector for delivery to the targeted cells
10   (*e.g.*, Johnson, International Publication No. WO95/34670;
International Application No. PCT/US95/07178). The
recombinant adeno-associated virus (AAV) genome typically
contains AAV inverted terminal repeats flanking a DNA sequence
encoding a TNFr/OPG-like polypeptide operably linked to
15   functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not
limited to, retrovirus, adenovirus, herpes simplex virus,
lentivirus, hepatitis virus, parvovirus, papovavirus,
poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus,
20   and papilloma virus vectors. U.S. Patent No. 5,672,344
describes an *in vivo* viral-mediated gene transfer system
involving a recombinant neurotrophic HSV-1 vector. U.S.
Patent No. 5,399,346 provides examples of a process for
providing a patient with a therapeutic protein by the delivery
25   of human cells which have been treated *in vitro* to insert a
DNA segment encoding a therapeutic protein. Additional
methods and materials for the practice of gene therapy
techniques are described in U.S. Patent No. 5,631,236
involving adenoviral vectors; U.S. Patent No. 5,672,510
30   involving retroviral vectors; and U.S. 5,635,399 involving
retroviral vectors expressing cytokines.

Nonviral delivery methods include, but are not limited
to, liposome-mediated transfer, naked DNA delivery (direct

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injection), receptor-mediated transfer (ligand-DNA complex),
electroporation, calcium phosphate precipitation, and
microparticle bombardment (e.g., gene gun). Gene therapy
materials and methods may also include the use of inducible
5   promoters, tissue-specific enhancer-promoters, DNA sequences
designed for site-specific integration, DNA sequences capable
of providing a selective advantage over the parent cell,
labels to identify transformed cells, negative selection
systems and expression control systems (safety measures),
10   cell-specific binding agents (for cell targeting), cell-
specific internalization factors, and transcription factors to
enhance expression by a vector as well as methods of vector
manufacture. Such additional methods and materials for the
practice of gene therapy techniques are described in U.S.
15   Patent No. 4,970,154 involving electroporation techniques;
WO96/40958 involving nuclear ligands; U.S. Patent No.
5,679,559 describing a lipoprotein-containing system for gene
delivery; U.S. Patent No. 5,676,954 involving liposome
carriers; U.S. Patent No. 5,593,875 concerning methods for
20   calcium phosphate transfection; and U.S. Patent No. 4,945,050
wherein biologically active particles are propelled at cells
at a speed whereby the particles penetrate the surface of the
cells and become incorporated into the interior of the cells.

In yet other embodiments, regulatory elements can be
25   included for the controlled expression of the TNFr/OPG-like
gene in the target cell. Such elements are turned on in
response to an appropriate effector. In this way, a
therapeutic polypeptide can be expressed when desired. One
conventional control means involves the use of small molecule
30   dimerizers or rapalogs (as described in WO9641865
(PCT/US96/099486); WO9731898 (PCT/US97/03137) and WO9731899
(PCT/US95/03157)) used to dimerize chimeric proteins which
contain a small molecule-binding domain and a domain capable
of initiating biological process, such as a DNA-binding

protein or transcriptional activation protein. The
dimerization of the proteins can be used to initiate
transcription of the transgene.

    An alternative regulation technology uses a method of
5   storing proteins expressed from the gene of interest inside
the cell as an aggregate or cluster. The gene of interest is
expressed as a fusion protein that includes a conditional
aggregation domain which results in the retention of the
aggregated protein in the endoplasmic reticulum. The stored
10   proteins are stable and inactive inside the cell. The
proteins can be released, however, by administering a drug
(e.g., small molecule ligand) that removes the conditional
aggregation domain and thereby specifically breaks apart the
aggregates or clusters so that the proteins may be secreted
15   from the cell. See, Science 287:816-817, and 826-830 (2000).

    Other suitable control means or gene switches include,
but are not limited to, the following systems. Mifepristone
(RU486) is used as a progesterone antagonist. The binding of
a modified progesterone receptor ligand-binding domain to the
20   progesterone antagonist activates transcription by forming a
dimer of two transcription factors which then pass into the
nucleus to bind DNA. The ligand binding domain is modified to
eliminate the ability of the receptor to bind to the natural
ligand. The modified steroid hormone receptor system is
25   further described in U.S. 5,364,791; WO9640911, and WO9710337.

    Yet another control system uses ecdysone (a fruit fly
steroid hormone) which binds to and activates an ecdysone
receptor (cytoplasmic receptor). The receptor then
translocates to the nucleus to bind a specific DNA response
30   element (promoter from ecdysone-responsive gene). The
ecdysone receptor includes a transactivation domain/DNA-
binding domain/ligand-binding domain to initiate
transcription. The ecdysone system is further described in
U.S. 5,514,578; WO9738117; WO9637609; and WO9303162.

Another control means uses a positive tetracycline-
controllable transactivator. This system involves a mutated
tet repressor protein DNA-binding domain (mutated tet R-
4 amino acid changes which resulted in a reverse tetracycline-
5   regulated transactivator protein, i.e., it binds to a tet
operator in the presence of tetracycline) linked to a
polypeptide which activates transcription. Such systems are
described in U.S. Patent Nos. 5,464,758; 5,650,298 and
5,654,168.

10   Additional expression control systems and nucleic acid
constructs are described in U.S. Patent Nos. 5,741,679 and
5,834,186 to Innovir Laboratories Inc.

It is also contemplated that TNFr/OPG-like molecule gene
therapy or cell therapy can further include the delivery of a
15   second polypeptide. For example, the host cell may be
modified to express and release both TNFr/OPG-like polypeptide
and at least one of the following: IL-1ra, sTNFr Type I, sTNFr
Type II, and derivatives thereof; Serine Leukocyte Protease
Inhibitor (SLPI), Osteoprotogerin (OPG); and anti-TNF
20   antibodies, anti-IL-1 antibodies, and derivatives thereof.
Alternatively, the TNFr/OPG-like polypeptide and one or more
of the above polypeptides may be expressed in and released
from separate cells. Such cells may be separately introduced
into the patient, or the cells may be contained in a single
25   implantable device, such as the encapsulating membrane
described above, or the cells may be separately modified by
means of viral vectors.

One example of a gene therapy technique is to use the
TNFr/OPG-like gene (either genomic DNA, cDNA, and/or synthetic
30   DNA encoding a TNFr/OPG-like polypeptide which may be operably
linked to a constitutive or inducible promoter to form a "gene
therapy DNA construct". The promoter may be homologous or
heterologous to the endogenous TNFr/OPG-like gene, provided
that it is active in the cell or tissue type into which the

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construct will be inserted. Other components of the gene
therapy DNA construct may optionally include, DNA molecules
designed for site-specific integration (e.g., endogenous
sequences useful for homologous recombination), tissue-
5   specific promoter, enhancer(s) or silencer(s), DNA molecules
capable of providing a selective advantage over the parent
cell, DNA molecules useful as labels to identify transformed
cells, negative selection systems, cell specific binding
agents (as, for example, for cell targeting), cell-specific
10   internalization factors, and transcription factors to enhance
expression by a vector as well as factors to enable vector
manufacture.

     This gene therapy DNA construct can then be introduced
into cells (either ex vivo or in vivo). One means for
15   introducing the gene therapy DNA construct is by means of
viral vectors as described herein. Certain vectors, such as
retroviral vectors, will deliver the gene therapy DNA
construct to the chromosomal DNA of the cells, and the gene
therapy DNA construct can integrate into the chromosomal DNA.
20   Other vectors will function as episomes, and the gene therapy
DNA construct will remain in the cytoplasm.

     Another means to increase endogenous TNFr/OPG-like
polypeptide expression in a cell via gene therapy is to insert
one or more enhancer elements into the TNFr/OPG-like
25   polypeptide promoter, where the enhancer element(s) can serve
to increase transcriptional activity of the TNFr/OPG-like
gene. The enhancer element(s) used will be selected based on
the tissue in which one desires to activate the gene(s);
enhancer elements known to confer promoter activation in that
30   tissue will be selected. For example, if a gene encoding a
TNFr/OPG-like polypeptide is to be "turned on" in T-cells, the
lck promoter enhancer element may be used. Here, the
functional portion of the transcriptional element to be added
may be inserted into a fragment of DNA containing the

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TNFr/OPG-like polypeptide promoter (and optionally, inserted into a vector and/or 5' and/or 3' flanking sequence(s), etc.) using standard cloning techniques. This construct, known as a "homologous recombination construct", can then be introduced

5   into the desired cells either *ex vivo* or *in vivo*.

Gene therapy can be used to decrease TNFr/OPG-like polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For

10   example, a DNA molecule containing all or a portion of the promoter of the TNFr/OPG-like gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example the TATA box and/or the binding site of a transcriptional

15   activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the corresponding TNFr/OPG-like gene. The deletion of the TATA box or the transcription activator binding site in the

20   promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the TNFr/OPG-like polypeptide promoter(s) (from the same or a related species as the TNFr/OPG-like gene(s) to be regulated) in which one or more of the TATA box and/or transcriptional activator binding

25   site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. This construct, which also will typically contain at least about 500 bases of DNA that

30   correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified, may be introduced into the appropriate cells (either *ex vivo* or *in vivo*) either directly or via a viral vector as described herein. Typically, the integration of the construct into the

genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

5   Other gene therapy methods may also be employed where it is desirable to inhibit the activity of one or more TNFr/OPG-like polypeptides. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of the selected TNFr/OPG-like gene(s) can be

10   introduced into the cell. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected TNFr/OPG-like gene. When the antisense molecule then hybridizes to the corresponding TNFr/OPG-like mRNA, translation of this mRNA is prevented or reduced. It will

15   also be appreciated by those skilled in the art that antisense and ribozyme molecules may also be administered directly.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more TNFr/OPG-like polypeptides. In this situation, the DNA encoding a mutant

20   full length or truncated polypeptide of each selected TNFr/OPG-like polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

25

Additional Uses of TNFr/OPG-Like Nucleic Acids and Polypeptides

Nucleic acid molecules of the present invention may be used to map the locations of the TNFr/OPG-like gene and

30   related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization.

The nucleic acid molecules are also used as anti-sense inhibitors of TNFr/OPG-like polypeptide expression. Such

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inhibition may be effected by nucleic acid molecules which are
complementary to and hybridize to expression control sequences
(triple helix formation) or to TNFr/OPG-like mRNA. Anti-sense
probes may be designed by available techniques using the

5   sequence of TNFr/OPG-like nucleic acid molecules disclosed
herein. Anti-sense inhibitors provide information relating to
the decrease or absence of a TNFr/OPG-like polypeptide in a
cell or organism.

Hybridization probes may be prepared using the TNFr/OPG-

10   like nucleic acid sequences provided herein to screen cDNA,
genomic or synthetic DNA libraries for related sequences.
Regions of the DNA and/or amino acid sequence of TNFr/OPG-like
polypeptide that exhibit significant identity to known
sequences are readily determined using sequence alignment

15   algorithms as described herein and those regions may be used
to design probes for screening.

TNFr/OPG-like nucleic acid molecules, as well as
fragments, variants, and/or derivatives that do not themselves
encode biologically active polypeptides, may be useful as

20   hybridization probes in diagnostic assays to test, either
qualitatively or quantitatively, for the presence of TNFr/OPG-
like DNA or corresponding RNA in mammalian tissue or bodily
fluid samples.

The TNFr/OPG-like polypeptides may be used

25   (simultaneously or sequentially) in combination with one or
more cytokines, growth factors, antibiotics, anti-
inflammatories, and/or chemotherapeutic agents as is
appropriate for the indication being treated.

TNFr/OPG-like polypeptide fragments, variants, and/or

30   derivatives, whether biologically active or not, are also
useful for preparing antibodies that bind to a TNFr/OPG-like
polypeptide. The antibodies may be used for *in vivo* and *in
vitro* diagnostic purposes, including, but not limited to, use
in labeled form to detect the presence of TNFr/OPG-like

polypeptide in a body fluid or cell sample. The antibodies
may also be used to prevent or treat the diseases and
disorders recited herein. The antibodies may bind to a
TNFr/OPG-like polypeptide so as to diminish or block at least
5   one activity characteristic of a TNFr/OPG-like polypeptide, or
may bind to a polypeptide to increase an activity.

The following example will serve to further typify the
nature of the invention but should not be construed as a
limitation on the scope thereof which is defined solely by the
10   appended claims.

EXAMPLE 1
Cloning of TNFr/OPG-like cDNA

Homology-based BLAST searches of a human genomic database
15   identified a 543 nucleotide genomic DNA fragment (SEQ ID NO:
5) which upon translation displayed homology to the known
human OPG polypeptide sequence. Based upon this sequence
information, nucleotide primers 2374-51 (5'- CCC CAG GCA CCT
TCT CAG CTG C - 3' SEQ ID NO: 9) and 2374-52 (5' - GTG TAT CTC
20   GAG TTG CCA TGC CC -3'; SEQ ID NO: 10) were synthesized and
used to screen a variety of human cDNA libraries. Using PCR
beads (Pharmacia, Piscataway, NJ), a final reaction volume of
25µl, and 10 pmol of each oligonucleotide, the expected size
band of 111 nucleotides (nt) was identified in a number of
25   libraries including fetal scalp (both random and oligo dT
primed) and fetal spleen (both random and oligo dT primed. The
cycling conditions were 94°C for 1 min, (94°C for 30 sec, 68°C
for 45 sec) repeat 35 times, then 72°C for 10 minutes.

Based upon this, to isolate the 5' region of the cDNA,
30   PCR was performed on the fetal spleen and fetal scalp cDNA
libraries using pSPORT (LTI) vector primers 870-02 (5 - AGC
GGA TAA CAA TTT CAC ACA GG - 3'; SEQ ID NO: 11) and 1916-83 (5
- GGC TCG TAT GTT GTG TGG AAT TGT GAG CG - 3'; SEQ ID NO: 12),

and a gene specific primer 2374-53 (5'-CCC AGG CCA GCA GTC

TCC ACA G -3'; SEQ ID NO: 13) using Clontech Advantage PCR Mix

(Clontech, Palo alto, CA). The cycling conditions were as

follows: 94°C for 1 min; 94°C for 5 sec; 72°C for 3 min;

5   (Repeated 5 times); followed by: 94°C for 5 sec; 70°C for 3

min; (Repeated 5 times; followed by: 94°C for 5 sec; 68°C for 3

min; Repeated 25 times; 72°C for 10 min. PCR products were

obtained from these cDNA libraries.

The PCR products obtained in these reactions were diluted

10   1:100 and PCR amplified with nested vector primers 1019-06

(5'- GCT CTA ATA CGA CTC ACT ATA GGG -3'; SEQ ID NO: 14) and

1916-82 (5' - CAT GAT TAC GCC AAG CTC TAA TAC GAC TC - 3'; SEQ

ID NO: 15), and a nested gene specific primer 2374-52 (5' -

GTG TAT CTC GAG TTG CCA TGC CC - 3'; SEQ ID NO: 10). The

15   specific PCR products were subcloned into pGEM-T (Promega,

Madison, WI) using the TA cloning protocol according to the

manufacturer's instructions. The 3' region was isolated by

PCR amplification of fetal scalp cDNA library using a vector

primers 1340-35 (5' - CCC AGT CAC GAC GTT GTA AAA CG - 3': SEQ

20   ID NO: 16) and a gene specific primer 2374-51 (5' - CCC CAG

GCA CCT TCT CAG CTG C - 3'; SEQ ID NO: 9) using Clontech

Advantage PCR Mix (Clontech, Palo alto, CA). The cycling

conditions were 94°C for 2 min, (94°C for 15 sec, 66°C for 15

sec, and 72°C for 3 min) repeated 35 times, 72°C for 2 min and

25   then kept at 4°C until being analyzed. The PCR products

obtained in the reaction were diluted 1:100 and PCR amplified

with a nested vector primer 1019-05 (5' - TGA ATT TAG GTG ACA

CTA TAG AAG AG - 3': SEQ ID NO: 17) and a nested gene specific

primer 2374-78 (5' - GCC CGT TGC AGC CTT TGG AG - 3': SEQ ID

30   NO: 18) using Clontech Advantage PCR Mix (Clontech, Palo alto,

CA). The cycling conditions were the same as mentioned above.

The final PCR products were subcloned into pGEM-T (Promega,

Madison, WI) using the TA cloning protocol. The sequence of

the 5' RACE clones and 3' region was determined by DNA
sequencing using standard methods known to those skilled in
the art.   The sequence was assembled and found to encode a
protein of 430 amino acids in length.

5   The cDNA libraries utilized to isolate this TNFr/OPG-like
gene were made as follows.   Total RNA was extracted from human
tissue using standard RNA extraction procedures and poly-A$^+$
RNA was selected from this total RNA using standard procedures
known to those skilled in the art.   Random primed or oligo(dT)

10   primed cDNA was synthesized from this poly-A$^+$ RNA using the
procedure in the manual of the Superscript Plasmid System for
cDNA Synthesis and Plasmid Cloning kit (Gibco-BRL, Inc.,
Rockville, MD) or using other suitable procedures known to
those skilled in the art.   The resulting cDNA was digested

15   with appropriate restriction enzymes (SalI and NotI) to create
sticky ends to assist in ligation to a cloning vector.   This
digested cDNA was then ligated into the pSPORT-1 cloning
vector, or another suitable cloning vector known to those
skilled in the art, that had been pre-digested with

20   appropriate restriction enzymes.   The ligation products were
transformed into E. coli using standard techniques known in
the art, and transformants were selected on bacterial media
plates containing ampicillin.   The cDNA library consisted of
all, or a subset, of these transformants.

25

EXAMPLE 2

Evaluation of TNFr/OPG Tissue Expression

Methods for mRNA expression analysis by RT-PCR were as
follows.

30   Reverse transcription (RT) reactions.   2ug of total RNA
from each human fetal tissue (total RNAs were purified by
Total RNA Isolation Kit from Amersham Pharmacia Biotech Inc.,

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Cat.# 15593-031). The reaction Mixture contained 2 ug total
RNA, and 1 ul (1 ug) Random Primer. The volume was adjusted
to 12 ul with water, heated to 70°C for 10 min, and quick-
chilled on ice. 4 ul 5xFirst Stand Buffer (BRL), 2 ul 0.1 M

5   DTT (BRL), and 1 ul 10 mM dNTP Mix(BRL) were then added, and
the solution was mixed well and warmed to 37°C for 2 min. 1
ul Superscript II RT (BRL) was added, and the solution was
incubated at 37°C for 1 hr.

The reaction tube was then placed in ice to terminate the

10 reaction. cDNAs produced in this way were used as the
template in the PCR analysis.

Estimate of relative expression levels

In order to normalize for differences in RNA

15 concentration and cDNA conversion efficiency, control PCRs
were performed on each cDNA using primers to Glyceraldehyde-3-
phosphate dehydrogenase (G3PDH), a gene expected to be
expressed at about the same level in all tissues. The
products of this reaction were analyzed on 4% agarose gels and

20 the relative intensity of the control bands were estimated.
cDNA samples were then diluted according to the intensity of
the control bands so that all samples were adjusted to a
concentration that would produce G3PDH control bands of equal
intensity. Expression analysis for the OPG-like transcript

25 was done using these concentration-normalized samples.

G3PDH control PCRs

Template: 1μl of cDNA (prior to concentration adjustment)
Primers:5' primer:5'-TCCACCACCCTGTTGCTGTAG-3' SEQ ID NO: 19

30   3' primer:5'-GACCACAGTCCATGCCATCACT-3'SEQ ID NO: 20

Buffer/enzyme: Ready-To-Go PCR Beads (Amersham Pharmacia
Biotech Inc., Cat. # 27-95530)
Cycling protocol:

35

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```
95°C 60 sec;
92°C 30 sec, 55°C 45 sec, 72°C 60 sec, 25 cycles;
72°C 5 min.
```

5   Relative expression levels of OPG-like transcript

Template: 1µl of cDNA (concentration adjusted as described above).

Primers: (2374-51) 5'-CCCCAGGCACCTTCTCAGCTGC-3' SEQ ID NO: 9
(2374-53) 5'-CCCAGGCCAGCAGTCTCCACAG-3' SEQ ID NO: 13

10   Buffer/enzyme: Ready-To-GO PCR Beads from Amersham Pharmacia Biotech Inc. (Cat. # 27-95530).

Cycling protocol:

```
95°C 30 sec;
94°C 5 sec, 72°C 4 min, 5 cycles;
94°C 5 sec, 70°C 4 min,  5 cycles;
94°C 5 sec, 68°C 2 min,  25 cycles;
72°C 3 min.
```

Products were run on 4% agarose/TBE gel electrophoresis. Using the faintest band as a baseline (1X), the relative
20   intensity of the band corresponding to the amplified OPG-like transcript was then estimated. The estimated relative intensity of each band is set forth below, with highest intensities being found in fetal tissue, fetal uterus and fetal skin.

25

#	Tissue	Expression Level
1	Fetal Stomach	XXX
2	Fetal Pancreas	XXX
3	Fetal Bladder	XXX
4	Fetal Brain	XXX
5	Lymphoma Cell Lines	XX
6	Fetal Testis	XXXXX
7	Fetal Thymus	XXXX
8	Fetal Placenta	XX
9	Fetal Spinal Cord	XXX
10	Fetal Heart	X
11	Fetal Uterus	XXXXX
12	Fetal Kidney	XXXX
13	Fetal Skin	XXXXX
14	Fetal Liver	XXX
15	Fetal Lung	XXX
16	Fetal Mesentery	XX
17	Fetal Bone	XX

5   EXAMPLE 3

            Production of TNFr/OPG-like polypeptides

A. Bacterial Expression

            PCR is used to amplify template DNA sequences
   encoding a polypeptide using primers corresponding to the 5'
10   and 3' ends of the sequence. The amplified DNA products may be
   modified to contain restriction enzyme sites to allow for
   insertion into expression vectors. PCR products are gel
   purified and inserted into expression vectors using standard
   recombinant DNA methodology. An exemplary vector, such as
15   pAMG21 (ATCC No. 98113) containing the lux promoter and a gene
   encoding kanamycin resistance is digested with BamHI and NdeI
   for directional cloning of inserted DNA. The ligated mixture
   is transformed into an *E. coli* host strain by electroporation
   and transformants are selected for kanamycin resistance.

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Plasmid DNA from selected colonies is isolated and subjected
to DNA sequencing to confirm the presence of the insert.

    Transformed host cells are incubated in 2xYT medium
containing 30 g/ml kanamycin at 30oC prior to induction.

5   Gene expression is induced by the addition of N-(3-
oxohexanoyl)-dl-homoserine lactone to a final concentration of
30 ng/ml followed by incubation at either 30oC or 37oC for six
hours. The expression of TNFr/OPG-like polypeptide is
evaluated by centrifugation of the culture, resuspension and

10 lysis of the bacterial pellets, and analysis of host cell
proteins by SDS-polyacrylamide gel electrophoresis.

    Inclusion bodies containing TNFr/OPG-like
polypeptide are purified as follows. Bacterial cells are
pelleted by centrifugation and resuspended in water. The cell

15 suspension is lysed by sonication and pelleted by
centrifugation at 195,000xg for 5 to 10 minutes. The
supernatant is discarded, and the pellet is washed and
transferred to a homogenizer. The pellet is homogenized in 5
ml of a Percoll solution (75% liquid Percoll. 0.15M NaCl)

20 until uniformly suspended and then diluted and centrifuged at
21,600xg for 30 minutes. Gradient fractions containing the
inclusion bodies are recovered and pooled. The isolated
inclusion bodies are analyzed by SDS-PAGE.

    A single band on an SDS polyacrylamide gel

25 corresponding to E. coli produced TNFr/OPG-like polypeptide is
excised from the gel, and the N-terminal amino acid sequence
is determined essentially as described by Matsudaira et al.,
J. Biol. Chem., 262:10-35 (1987).

30 B. Mammalian Cell Production

    PCR is used to amplify template DNA sequences
encoding a TNFr/OPG-like polypeptide using primers
corresponding to the 5' and 3' ends of the sequence. The
primer sequences corresponding to the 5' and 3' ends are

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described above. The amplified DNA products may be modified
to contain restriction enzyme sites to allow for insertion
into expression vectors. PCR products are gel purified and
inserted into expression vectors using standard recombinant

5   DNA methodology. An exemplary expression vector, pCEP4
(Invitrogen, Carlsbad, CA), which contains an Epstein-Barr
virus origin of replication, may be used for the expression of
TNFr/OPG-like in 293-EBNA-1 (Epstein-Barr virus nuclear
antigen) cells. Amplified and gel purified PCR products are

10   ligated into pCEP4 vector and lipofected into 293-EBNA cells.
The transfected cells are selected in 100 g/ml hygromycin and
the resulting drug-resistant cultures are grown to confluence.
The cells are then cultured in serum-free media for 72 hours.
The conditioned media is removed and, TNFr/OPG-like

15   polypeptide expression is analyzed by SDS-PAGE.
TNFr/OPG-like polypeptide expression may be detected
by silver staining. Alternatively, TNFr/OPG-like polypeptide
is produced as a fusion protein with an epitope tag, such as
an IgG constant domain or a FLAG epitope, which may be

20   detected by Western blot analysis using antibodies to the tag
peptide.
TNFr/OPG-like polypeptides may be excised from an
SDS-polyacrylamide gel, or TNFr/OPG-like fusion proteins are
purified by affinity chromatography to the epitope tag, and

25   subjected to N-terminal amino acid sequence analysis as
described herein.

EXAMPLE 4

Production of Anti-TNFr/OPG-like Polypeptide Antibodies

30   Antibodies to TNFr/OPG-like polypeptides may be
obtained by immunization with purified protein or with
TNFr/OPG-like peptides produced by biological or chemical
synthesis. Suitable procedures for generating antibodies
include those described in Hudson and Hay, Practical

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Immunology, 2nd Edition, Blackwell Scientific Publications
(1980).

In one procedure for the production of antibodies,
animals (typically mice or rabbits) are injected with a
5   TNFr/OPG-like antigen (such as a TNFr/OPG-like polypeptide),
and those with sufficient serum titer levels as determined by
ELISA are selected for hybridoma production. Spleens of
immunized animals are collected and prepared as single cell
suspensions from which splenocytes are recovered. The
10   splenocytes are fused to mouse myeloma cells (such as Sp2/0-
Ag14 cells; ATCC no. CRL-1581), allowed to
incubate in DMEM with 200 U/ml penicillin, 200 g/ml
streptomycin sulfate, and 4 mM glutamine, then incubated in
HAT selection medium (Hypoxanthine; Aminopterin; Thymidine).
15   After selection, the tissue culture supernatants are taken
from each well containing a hybridoma and tested for anti-
TNFr/OPG-like antibody production by ELISA.

Alternative procedures for obtaining anti-TNFr/OPG-
like antibodies may also be employed, such as the immunization
20   of transgenic mice harboring human Ig loci for the production
of human antibodies, and the screening of synthetic antibody
libraries, such as those generated by mutagenesis of an
antibody variable domain.

25   **EXAMPLE 5**

Production of TNFr/OPG-like Protein in Mammalian Cells

To generate soluble TNFr/OPG-like protein in mammalian
cells, the cDNA encoding the extracellular domain of human
TNFr/OPG-like polypeptide (amino acid 1-162) was PCR amplified
30   with the following set of oligo primer pair:

5' -CCA TCG ATG GCT GAG CAG CAG GTG TGG ACA-3' (SEQ ID NO: 21)

5' -TGG CGA TGA CGG TGA CCT GGG CGG-3' (SEQ ID NO: 22).

The PCR reaction was carried out in a 50 µl volume which
consisted of 1 unit of vent DNA polymerase (New England

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Biolabs) in 20 mM Tris-HCl pH 8.8, 10 mM KCl, 10 µM (NH$_4$)$_2$SO$_4$,
0.1% Triton-X100, 10 µM of each dNTP, 1µM of each primer and
10 ng of TNFr/OPG-like cDNA template. PCR Reactions were
performed at 98°C for 30 seconds, 55°C for 30 seconds, and 72°C
5   for 1 minute, for a total of 5 cycles and 98° for 30 seconds,
65° for 30 seconds, 72° for 1 minute for a total of 25 cycles.
The resulting PCR fragment was isolated by electrophoresis
through 1% agarose gel and purification by the Geneclean
procedure (Bio 101, Inc.). The PCR fragment creates a Cla I
10   restriction site at its 5' end and a BstEII restriction site
at its 3' end. The ClaI+BstEII digested PCR fragment was then
subcloned in-frame into a modified pCMVi-Fc vector in front of
the human IgG-γ1 heavy chain sequence as described previously
by Vasser et al. (Science 286, pp. 735-741, 1999). A linker
15   was introduced which encodes two irrelevant amino acids (Val-
Thr) spanning the junction between the TNFr/OPG-like
extracellular domain and the IgG Fc region.

The construct was transfected into 293-T cells by the
20   calcium phosphate method as described by Ausubel et al. (Curr.
Prot. Mol. Biol. 1, 9.1.1-9.1.3, 1994). Twenty-four hours
post-transfection, the cells were washed in PBS once and then
cultured in serum-free media for 72 hr. The conditioned media
was collected. The TNFr/OPG-like-Fc fusion protein that was
25   secreted into the media was detected by Western blot analysis
with anti-human IgG Fc antibody (Jackson Immuno Reasearch cat
no. 309-035-008) (Figure 9) and three distinct bands were
observed having molecular weights, 56.6 kD, 44.3kD, and 40.6
kD respectively.
30

The Fc fusion protein was purified by protein-A column
chromatography (Pierce) according to the manufacturer's
recommended procedures. Fifty pmoles of the purified protein
was then subjected to N-terminal sequence analysis by

automated Edman degradation as essentially described by
Matsudaira et al. (J. Biol. Chem. 262, 10-35, 1987).

    Following 10 cycles of amino acid sequencing, the 56.6
5   kD band gave the sequence NH₂- ST(T)LWQCPPGEE-CO₂H (1.4
    pmol)(SEQ ID NO: 23). At the third cycle, Thr was not
    detected as expected from the primary structure of the
    protein, indicating the possibility of O-linked sugars. The
    results show that the protein was cleaved at Thr25. The 44.3
10 kD band (14.6 pmol) and the 40.6 kD band (24.7 pmol) both gave
    the sequence NH₂-GVEVAAGASSGGET-CO₂H (SEQ ID NO: 24);
    indicating the protein was cleaved at Arg130. The difference
    in size between these two bands is presumably due to
    differential N-linked glycosylation at Arg149. The 40.6 kD
15 band and the 44.3 kD band represent approximately 97 % of the
    recovered material. Closer examination of the cleavage site
    at Arg 130 reveals a consensus furin cleavage site beginning
    with Arg126 (RRARR-GVEV…) (SEQ ID NO: 25).
        To explore the role of furin in the cleavage of
20 TNFr/OPG-like receptor extracellular domain, we transiently
    transfected 293-T cells with TNFr/OPG-like-Fc with or without
    co-transfection of the potent furin inhibitor α1-antitrypsin
    containing the Portland mutation (α1-PDX)   (J. Biol. Chem.:
    24887-91. 1993).   In short, 7x10⁶ 293-T cells were
25 transiently transfected with 20 µgs of TNFr/OPG-like-Fc alone
    or with 15 µgs of TNFr/OPG-like-Fc and 5 µgs of α1-PDX, using
    the CaOPO₄ method of transfection described above. The
    conditioned medium was collected and subjected to Western blot
    analysis as described above. Co-transfection with α1-PDX
30 completely abrogated furin cleavage resulting in 100% of the
    recovered material beginning with Ser26 as shown in Fiure 9
    (left panel).
        To further confirm the role of furin cleavage in
    liberation of a soluble extracellular domain of TNFr/OPG-like

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receptor, we engineered a version of the TNFr/OPG-like
receptor containing the signal peptide from OPG and an in
frame, NH2 terminal FLAG epitope tag (SO.FLAG-TNFr/OPG-like
receptor).   The SO.FLAG-TNFr/OPG-like receptor construct

5   encodes a protein containing OPG signal peptide (amino acid 1-
21)-linker(KLH)-FLAG epitope (MDYKDDDDK; SEQ ID NO: 26)-
linker(KL)-TNFr/OPG-like receptor (amino acid 26-430).
Again, $7x10^6$ 293-T cells were transfected with SO.FLAG-
TNFr/OPG-like receptor alone or co-transfected with α1-PDX.

10   Twnet-four hours after the transfection, cells were incubated
in serum free media for 72 hours. The conditioned media was
collected and analyzed by immunoprecipitation/Western blotting
using the anti-FLAG monoclonal antibody M2 (Sigma, St.Louis
MO). Two distinct bands of 17 KDa and 18 Kda were detected in

15   the conditioned medium, corresponding to the cleaved soluble
extracellular domain of OPG-like receptor as shown in Figure 9
(left panel). Similarly co-transfection with α1-PDX
dramatically reduces the amount of shed FLAG-TNFr/OPG-like
extracellular domain recovered from the conditioned media.

20

EXAMPLE 6

Detection of TNFr/OPG-like-Fc Binding to WEHI-3 Cells

The binding activity of TNFr/OPG-like-Fc with various
cell lines was tested by FACS analysis as previously described

25   (Goodwin et al. Cell, 73, 447-456, 1993). Briefly, WEHI-3
cells were incubated for 30 minutes at 4° C in PBS
supplemented with 2% rabbit serum and 5% goat serum for
blocking purposes. Subsequently, the cells were incubated
with 1 μg/ml TNFr/OPG-like-Fc fusion protein or human IgG.

30   The cells were then stained for 30 minutes at 4° C with
biotinylated antibody specific for the Fc domain of human IgG
(Jackson Immunoresearch,West Grove, PA) at a dilution of
1:200, followed by a 30 minute incubation with streptavidin-
phycoerythrin (Jackson Immunoresearch,West Grove, PA)   at a

dilution of 1:50. The cells were then subjected to FACS
analysis using a Becton Dickenson FACS Scan. TAJ.FC and
E127.FC were non-specific fusion proteins used for controls
and did not result in any specific binding. This analysis
5   revealed that WEHI-3 cells, a myelo-monocytic cell line,
specifically bound the TNFr/OPG-like-Fc fusion protein
indicating the presence of a membrane bound form of a putative
ligand for the OPGlike receptor.(See Figure 10) The binding
of TNFr/OPG-like-Fc fusion protein was partially blocked by
10   pre-incubation with conditioned media containing the N-
terminal FLAG tagged TNFr/OPG-like extracellular domain.

EXAMPLE 7

Northern Blot Analysis of TNFr/OPG-like receptor mRNA Tissue
15   **Expression**

Northern blot analysis was performed to identify those

tissues in which the TNFr/OPG-like receptor transcript is

expressed. A probe for use in Northern blot analysis was

20   generated by digesting the human TNFr/OPG-like receptor cDNA

with *EcoRV and XhoI* for about three hours at 37° C and

running the restriction digest on an 0.8% agarose gel to

separate the fragments. An approximately 434 base pair *ECORV-*

XhoI fragment, extending from nucleotide -180 to nucleotide

25   +254 of the cDNA,was isolated and gel purified using the

QiaQuick® gel purification system (Qiagen, Chatsworth, CA).

The isolated, gel purified fragment was quantitated by

estimation on a one percent agarose gel. About 25 ng of this

fragment was denatured by boiling for 5 minutes, and then

30   quenching on ice for 2 minutes. The fragment was then

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radioactively labeled with α-^{32}P-dCTP using the High Prime DNA

labeling kit (Boehringer Manheim, Indianapolis, IN) according

to the manufacturer's protocol. Human multiple tissue

northern blots were purchased (Clonetech, Palo Alto, CA) and

5   first prehybridized in Clontech Express™ hybridization buffer

for about one hour at about 65°C. Following prehbridization,

the labeled probe was denatured by boiling for about five

minutes then quenched on ice for 2 minutes, and added to the

hybridization buffer containing the Northern blots. The blots

10   were allowed to hybridize for about two hours at about 65°C.

After hybridization the blots were washed in 2xSSC for 30

minutes at room temperature, followed by 3 successive washes

in 0.2xSSC containing 0.1 percent SDS at about 60°C for 30

minutes. The blots were dried briefly and exposed to an image

15   analyzer screen for 6 days. The results are shown in Figure

11. TNFr/OPG-like receptor mRNA was mainly detected in

peripheral blood leukocytes, spleen, testis and skeletal

muscle.

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WHAT IS CLAIMED

1.   An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a)   the nucleotide sequence set forth in SEQ ID
5   NOS: 1 or 3;

(b)   a nucleotide sequence encoding the polypeptide set forth in SEQ ID NOS: 2 or 4;

(c)   a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of
10   (a) or (b), wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4; and

(d)   a nucleotide sequence complementary to any of (a)-(c).

15   2.   An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a)   a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide set forth in SEQ ID NOS: 2 or 4, wherein the polypeptide has
20   an activity of the polypeptide . set forth in SEQ ID NOS: 2 or 4;

(b)   a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence set forth in SEQ ID NOS: 1 or 3, wherein the encoded polypeptide
25   has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(c)   a nucleotide sequence of SEQ ID NOS: 1 or 3; (a); or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an
30   activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(d)   a nucleotide sequence of SEQ ID NOS: 1 or 3, or (a)-(c) comprising a fragment of at least about 16 nucleotides;

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(e)   a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4; and

5   (f)   a nucleotide sequence complementary to any of (a)-(c).

3.   An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

10   (a)   a nucleotide sequence encoding a polypeptide set forth in SEQ ID NOS: 2 or 4 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(b)   a nucleotide sequence encoding a polypeptide

15   set forth in SEQ ID NOS: 2 or 4 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(c)   a nucleotide sequence encoding a polypeptide set forth in SEQ ID NOS: 2 or 4 with at least one amino acid

20   deletion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(d)   a nucleotide sequence encoding a polypeptide set forth in SEQ ID NOS: 2 or 4 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity

25   of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(e)   a nucleotide sequence encoding a polypeptide set forth in SEQ ID NOS: 2 or 4 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-

30   terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;

(f)   a nucleotide sequence of (a)-(e) comprising a fragment of at least 16 nucleotides;

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(g)   a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NOS: 2 or 4; and

5   (h)   a nucleotide sequence complementary to any of (a)-(e).

4.   A vector comprising the nucleic acid molecule of claims 1, 2, or 3.

10

5.   A host cell comprising the vector of claim 4.

6.   The host cell of claim 5 that is a eukaryotic cell.

15   7.   The host cell of claim 5 that is a prokaryotic cell.

8.   A process of producing a TNFr/OPG-like polypeptide comprising culturing the host cell of claim 6 under suitable conditions to express the polypeptide, and optionally
20   isolating the polypeptide from the culture.

9   A polypeptide produced by the process of claim 8.

10.   The process of claim 11, wherein the nucleic acid
25   molecule comprises promoter DNA other than the promoter DNA for the native TNFr/OPG-like polypeptide operatively linked to the DNA encoding the TNFr/OPG-like polypeptide.

11.   The isolated nucleic acid molecule according to
30   claim 2 wherein the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

12.   A process for identifying candidate inhibitors of
TNFr/OPG-like polypeptide activity or production comprising
exposing a cell according to claims 6, 7, or 8 to the
candidate inhibitors, and measuring TNFr/OPG-like polypeptide
5   activity or production in said cell, comparing activity of
TNFr/OPG-like in cells exposed to the candidate inhibitor with
activity in cells not exposed to the candidate inhibitor.

13.   A process for identifying candidate stimulators of
10   TNFr/OPG-like polypeptide activity or production comprising
exposing a cell according to claims 6, 7, or 8 to the
candidate stimulators, and measuring TNFr/OPG-like polypeptide
activity or production in said cell, comparing activity of
TNFr/OPG-like in cells exposed to the candidate stimulator
15   with activity in cells not exposed to the candidate
stimulator.

14.   An isolated polypeptide comprising the amino acid
sequence set forth in SEQ ID NOS: 2 or 4.
20

15.   An isolated polypeptide comprising the amino acid
sequence selected from the group consisting of:
         (a)   the mature amino acid sequence set forth in
SEQ ID NOS: 2 or 4, comprising a mature amino terminus at
25   residue 1, optionally further comprising an amino-terminal
methionine;
         (b)   an amino acid sequence for an ortholog of SEQ
ID NOS: 2 or 4, wherein the encoded polypeptide has an
activity of the polypeptide set forth in SEQ ID NOS: 2 or 4;
30   (c)   an amino acid sequence that is at least about
70 percent identical to the amino acid sequence of SEQ ID NOS:
2 or 4, wherein the polypeptide has an activity of the
polypeptide set forth in SEQ ID NOS: 2 or 4;

(d)   a fragment of the amino acid sequence set forth
in SEQ ID NOS: 2 or 4 comprising at least about 25 amino acid
residues, wherein the polypeptide has an activity of the
polypeptide set forth in SEQ ID NOS: 2 or 4;

5   (e)   an amino acid sequence for an allelic variant
or splice variant of either the amino acid sequence set forth
in SEQ ID NOS: 2 or 4, or at least one of (a)-(c) wherein the
polypeptide has an activity of the polypeptide set forth in
SEQ ID NOS: 2 or 4.

10

16.   An isolated polypeptide comprising the amino acid
sequence selected from the group consisting of:

                  (a)   the amino acid sequence set forth in SEQ ID
NOS: 2 or 4 with at least one conservative amino acid

15   substitution, wherein the polypeptide has an activity of the
polypeptide set forth in SEQ ID NOS: 2 or 4;

                  (b)   the amino acid sequence set forth in SEQ ID
NOS: 3 OR 4 with at least one amino acid insertion, wherein
the polypeptide has an activity of the polypeptide set forth

20   in SEQ ID NOS: 2 or 4;

                  (c)   the amino acid sequence set forth in SEQ ID
NOS: 2 or 4 with at least one amino acid deletion, wherein the
polypeptide has an activity of the polypeptide set forth in
SEQ ID NOS: 2 or 4;

25   (d)   the amino acid sequence set forth in SEQ ID
NOS: 3 OR 4 which has a C- and/or N-terminal truncation,
wherein the polypeptide has an activity of the polypeptide
set forth in SEQ ID NOS: 2 or 4; and

                  (e)   the amino acid sequence set forth in SEQ ID
30   NOS: 2 or 4, with at least one modification selected from the
group consisting of amino acid substitutions, amino acid
insertions, amino acid deletions, C-terminal truncation, and
N-terminal truncation, wherein the polypeptide has an activity
of the polypeptide set forth in SEQ ID NOS: 2 or 4.

17.   An ortholog of claim 15 or 16 wherein the polypeptide is encoded by the polynucleotide set forth in SEQ ID NO: 6.

5

18.   A polypeptide according to claim 15 or 16 wherein the amino acid at position 42 of SEQ ID NOS: 2 is selected from the group consisting of glycine or proline.

10   19.   A polypeptide according to claim 15 or 16 wherein the amino acid at position 51 of SEQ ID NOS: 2 is selected from the group consisting of serine, threonine, aspargine, glutamine and cysteine.

15   20.   A polypeptide according to claim 15 or 16 wherein the amino acid at position 56 of SEQ ID NOS: 2 is selected from the group consisting of phenylalanine, tryptophan and tyrosine.

20   21.   A polypeptide according to claim 15 or 16 wherein the amino acid at position 68 of SEQ ID NOS: 2 is selected from the group consisting of lysine, arginine and histadine.

22.   A polypeptide according to claim 15 or 16 wherein
25 the amino acid at position 71 of SEQ ID NOS: 2 is selected from the group consisting of cysteine, serine, threonine, asparagine and glutamine.

23.   A polypeptide according to claim 15 or 16 wherein
30 the amino acid at position 84 of SEQ ID NOS: 2 is selected from the group consisting of leucine, norleucine, isoleucine, valine, methionine, alanine, norleucine or phenylalanine.

24. A polypeptide according to claim 15 or 16 wherein the amino acid at position 87 of SEQ ID NOS: 2 is selected from the group consisting of aspartic acid or glutamic acid.

5 25. An isolated polypeptide encoded by the nucleic acid molecule of claims 1, 2, or 3.

26. The isolated polypeptide according to claim 15 or 16 wherein the percent identity is determined using a computer
10 program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

27. An antibody produced by immunizing an animal with a
15 peptide comprising an amino acid sequence of SEQ ID NOS: 2 or 4.

28. An antibody or fragment thereof that specifically binds the polypeptide of claims 13, 14 or 15.
20

29. The antibody of claim 27 that is a monoclonal antibody.

30. A hybridoma that produces a monoclonal antibody that
25 binds to a peptide comprising an amino acid sequence of SEQ ID NOS: 2 or 4.

31. A method of detecting or quantitating the amount of TNFr/OPG-like in a sample comprising contacting a sample
30 suspected of containing TNFr/OPG-like polypeptide with the anti-TNFr/OPG-like antibody or fragment of claims 27, 28 or 29 and detecting the binding of said antibody or fragment.

32.   A selective binding agent or fragment thereof that specifically binds at least one polypeptide wherein said polypeptide comprises the amino acid sequence selected from the group consisting of:

5   (a)   the amino acid sequence set forth in SEQ ID NOS: 2 or 4;

              (b)   a fragment of the amino acid sequence set forth in at least one of SEQ ID NOS: 2 or 4; and

              (c) a naturally occurring variant of (a) or (b).

10

33.   The selective binding agent of claim 32 that is an antibody or fragment thereof.

34.   The selective binding agent of claim 32 that is a
15   humanized antibody.

35.   The selective binding agent of claim 32 that is a human antibody or fragment thereof.

20   36.   The selective binding agent of claim 32 that is a polyclonal antibody or fragment thereof.

37.   The selective binding agent claim 32 that is a monoclonal antibody or fragment thereof.

25   38.   The selective binding agent of claim 32 that is a chimeric antibody or fragment thereof.

39.   The selective binding agent of claim 32 that is a CDR-grafted antibody or fragment thereof.

30

40.   The selective binding agent of claim 32 that is an antiidiotypic antibody or fragment thereof.

41. The selective binding agent of claim 32 which is a variable region fragment.

42. The variable region fragment of claim 32 which is a
5   Fab or a Fab' fragment.

43. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for a polypeptide having the amino acid
10 sequence of SEQ ID NOS: 2 or 4.

44. The selective binding agent of claim 32 which is bound to a detectable label.

15   45. The selective binding agent of claim 32 which antagonizes TNFr/OPG-like polypeptide biological activity.

46. A method for treating, preventing, or ameliorating a disease, condition, or disorder associated with altered levels
20 of TNFr/OPG-like polypeptide comprising administering to a patient an effective amount of a selective binding agent according to claim 32.

47. A selective binding agent produced by immunizing an
25 animal with a polypeptide comprising an amino acid sequence of SEQ ID NOS: 2 or 4.

48. A hybridoma that produces a selective binding agent capable of binding a polypeptide according to claims 14, 15
30 or 16.

49. A composition comprising the polypeptide of claims 14, 15 or 16 and a pharmaceutically acceptable formulation agent.

50. The composition of claim 49 wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, anti-oxidant or
5 combination thereof.

51. The composition of claim 49 wherein the polypeptide comprises the mature amino acid sequence set forth in SEQ ID NOS: 2 or 4.
10

52. A polypeptide comprising a derivative of the polypeptide of claims 14, 15 or 16.

53. The polypeptide of claim 52 which is covalently
15 modified with a water-soluble polymer.

54. The polypeptide of claim 53 wherein the water-soluble polymer is selected from the group consisting of polyethylene glycol, monomethoxy-polyethylene glycol, dextran,
20 cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, and polyvinyl alcohol.

25   55. A composition comprising a nucleic acid molecule of claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

56. A composition of claim 55 wherein said nucleic acid
30 molecule is contained in a viral vector.

57. A viral vector comprising a nucleic acid molecule of claims 1, 2, or 3.

58.   A fusion polypeptide comprising the polypeptide of
claims 14, 15 or 16 fused to a heterologous amino acid
sequence.

5   59.   The fusion polypeptide of claim 58 wherein the
heterologous amino acid sequence is an IgG constant domain or
fragment thereof.

            60.   A method for treating, preventing or ameliorating a
10   medical condition in a mammal resulting from decreased levels
of TNFr/OPG-like polypeptide comprising administering to a
patient the polypeptide of claims 14, 15 or 16 or the
polypeptide encoded by the nucleic acid of claims 1, 2, or 3
to said mammal.

15

            61.   A method of diagnosing a pathological condition or a
susceptibility to a pathological condition in a subject caused
by or resulting from abnormal levels of TNFr/OPG-like
polypeptide comprising:

20   (a)   determining the presence or amount of
expression of the polypeptide of claims 14, 15 or 16 or the
polypeptide encoded by the nucleic acid molecule of claims 1,
2, or 3 in a sample; and
            (b)   comparing the level of TNFr/OPG-like
25   polypeptide in a biological, tissue or cellular sample from
normal subjects or the subject at an earlier time, wherein
susceptibility to a pathological condition is based on the
presence or amount of expression of the polypeptide.

30

62.   A device, comprising:

(a)   a membrane suitable for implantation; and

(b)   cells encapsulated within said membrane,

wherein said cells secrete a polypeptide of claims 14, 15 or

5   16, and wherein said membrane is permeable to said protein and

impermeable to materials detrimental to said cells.

63.   A device, comprising:

(a)   a membrane suitable for implantation; and

10   (b)   the TNFr/OPG-like polypeptide encapsulated

within said membrane, wherein said membrane is permeable to

the polypepetide.

64.   A method of identifying a compound which binds to a

15   polypeptide comprising:

(a)   contacting the polypeptide of claims 14, 15 or

16 with a compound; and

(b)   determining the extent of binding of the

polypeptide to the compound.

20

65.   A method of identifying antagonists of TNFr/OPG-like

biological activity comprising:

(a)   contacting a small molecule compound with a

TNFr/OPG-like polypeptide;

25   (b)   detecting the biological activity of TNFr/OPG-

like in the presence of said small molecule compound; and

(c)   comparing the level of TNFr/OPG-like biological

activity in the presence and absence of said small molecule

compound.

30

66.   The method of claim 65 wherein the small molecule

compound is a member of a naturally occurring chemical

library.

67. The method of claim 65 wherein the small molecule compound is a member of a naturally occurring medicinal chemical library.

5   68. The method of claim 65 wherein the small molecule compound is a member of a combinational chemical library.

69. A method of identifying a polypeptide which binds to a TNFr/OPG-like polypeptide, wherein the method utilizes a
10   yeast two-hybrid approach comprising:
              (a) preparing a bait construct comprising a GAL4 DNA binding domain·fused to the nucleotide sequence of claim 1, 2 or 3;
              (b) screening a cDNA library with the bait
15   construct, wherein said library consists of nucleotide sequences fused to a GAL4 activation domain, and
              (c) identifying polypeptides that bind to said construct by detecting transcriptional activation of a reporter gene under control of GAL4.
20

70. A TNFr/OPG-like polypeptide binding partner identified by the method of claim 71.

71.   A method of modulating levels of a polypeptide in
25   an animal comprising administering to the animal the nucleic acid molecule of claims 1, 2, or 3.

72. An antagonist of TNFr/OPG-like polypeptide activity selected from the group consisting of TNFr/OPG-like selective
30   binding agents, small molecules, antisense oligonucleotides, and peptides or derivatives thereof having specificity for TNFr/OPG-like polypeptide.

73.   A method of reducing cellular production of
TNFr/OPG-like, comprising transforming or transfecting cells
with an antagonist according to claim 72.

5   74.   A method according to claim 73, wherein the
antagonist is an antisense reagent, said reagent comprising an
oligonucleotide comprising a single stranded nucleic acid
sequence capable of binding to TNFr/OPG-like mRNA.

10   75.   A transgenic non-human mammal comprising the nucleic
acid molecule of claims 1, 2, or 3.

76.   A transgenic non-human mammal conprising a
disruption of the nucleic acid molecule of claim 1, 2 or 3
15 wherein the expression of TNFr/OPG-like polypeptide is
decreased.

77.   A diagnostic reagent comprising a detectably labeled
polynucleotide encoding the amino acid sequence set out in SEQ
20 ID NOS: 2 or 4, or a fragment, variant or homolog thereof
including allelic variants and spliced variants thereof.

78.   The diagnostic reagent of claim 77, wherein said
labeled polynucleotide is a first-strand cDNA.
25

79.   A method for determine the presence of TNFr/OPG-like
nucleic acids in a biological sample comprising the steps of:
(a)   providing a biological sample suspected of
containing TNFr/OPG-like nucleic acids;
30   (b)   contacting the biological sample with a
diagnostic reagent according to claim 84 under conditions
wherein the diagnostic reagent will hybridize with TNFr/OPG-
like nucleic acids contained in said biological sample;

(c)   detecting hybridization between nucleic acid in the biological sample and the diagnostic reagent; and

(d)   comparing the level of hybridization between the biological sample and diagnostic reagent with the level of

5   hybridization between a known concentration of TNFr/OPG-like nucleic acid and the diagnostic reagent.

80.   A method for detecting the presence of TNFr/OPG-like nucleic acids in a tissue or cellular sample comprising the

10   steps of:

(a)   providing a tissue or cellular sample suspected of containing TNFr/OPG-like nucleic acids;

(b)   contacting the tissue or cellular sample with a diagnostic reagent according to claim 77 under conditions

15   wherein the diagnostic reagent will hybridize with TNFr/OPG-like nucleic acids;

(c)   detecting hybridization between TNFr/OPG-like nucleic acid in the tissue or cellular sample and the diagnostic reagent; and

20   (d)   comparing the level of hybridization between the tissue or cellular sample and diagnostic reagent with the level of hybridization between a known concentration of TNFr/OPG-like nucleic acid and the diagnostic reagent.

25   81.   The method of claim 86 or 87 wherein said polynucleotide molecule is DNA.

82.   The method of claim 86 or 87 wherein said polynucleotide molecule is RNA.

30

Figure 1

cDNA Sequence of Human TNFr/OPG-Like Nucleic Acid Molecule

(SEQ ID NO. 1)

```
   1  CGGGACCTTC AGATATCCCC TCCCAGCCGA GGGGGCTTCC ATCTAACTGT

  51  TTTTTTGGTC ACGGTTCCAG GGCCGTTTTA GACAGTGGAG GCCTTGTGGG

 101  GCAGGGTGTG AGGGGTGCTG AGCAGCAGGT GTGGACATGT GTGTGCACCA

 151  GGCCTTTCTA CCTGACCGGG CCGGCGACCA CCAGGGGCCT GAGGATGAAG

 201  CCAAGTCTGC TGTGCCGGCC CCTGTCCTGC TTCCTTATGC TGCTGCCCTG

 251  GCCTCTCGCC ACCCTGACAT CAACAACCCT TTGGCAGTGC CCACCTGGGG

 301  AGGAGCCCGA CCTGGACCCA GGGCAGGGCA CATTATGCAG GCCCTGCCCC

 351  CCAGGCACCT TCTCAGCTGC ATGGGGCTCC AGCCCATGCC AGCCCCATGC

 401  CCGTTGCAGC CTTTGGAGGA GGCTGGAGGC CCAGGTGGGC ATGGCAACTC

 451  GAGATACACT CTGTGGAGAC TGCTGGCCTG GTGGTTTGG GCCTTGGGGG

 501  GTTCCCCGCG TTCCATGTCA ACCATGTTCC TGGGCACCTC TGGGTACTCA

 551  TGGCTGTGAT GAGTGGGGGC GGCGGGCCCG ACGTGGCGTG GAGGTGGCAG

 601  CAGGGGCCAG CAGCGGTGGT GAGACACGGC AGCCTGGGAA CGGCACCCGG

 651  GCAGGTGGCC CAGAGGAGAC AGCCGCCCAG TACGCGGTCA TCGCCATCGT

 701  CCCTGTCTTC TGCCTCATGG GGCTGTTGGG CATCCTGGTG TGCAACCTCC

 751  TCAAGCGGAA GGGCTACCAC TGCACGGCGC ACAAGGAGGT CGGGCCCGGC

 801  CCTGGAGGTG GAGGCAGTGG AATCAACCCT GCCTACCGGA CTGAGGATGC

 851  CAATGAGGAC ACCATTGGGG TCCTGGTGCG CTTGATCACA GAGAAGAAAG

 901  AGAATGCTGC GGCCCTGGAG GAGCTGCTGA AGAGTACCA CAGCAAACAG

 951  CTGGTGCAGA CGAGCCACAG GCCTGTGTCC AAGCTGCCGC CAGCGCCCCC

1001  GAACGTGCCA CACATCTGCC CGCACCGCCA CCATCTCCAC ACCGTGCAGG
```

Figure 1 (cont.)

```
1051    GCCTGGCCTC GCTCTCTGGC CCCTGCTGCT CCCGCTGTAG CCAGAAGAAG

1101    TGGCCCGAGG TGCTGCTGTC CCCTGAGGCT GTAGCCGCCA CTACTCCTGT

1151    TCCCAGCCTT CTGCCTAACC CGACCAGGGT TCCCAAGGCC GGGGCCAAGG

1201    CAGGGCGTCA GGGCGAGATC ACCATCTTGT CTGTGGGCAG GTTCCGCGTG

1251    GCTCGAATTC CTGAGCAGCG GACAAGTTCA ATGGTGTCTG AGGTGAAGAC

1301    CATCACGGAG GCTGGGCCCT CGTGGGGTGA TCTCCCTGAC TCCCCACAGC

1351    CTGGCCTCCC CCCTGAGCAG CAGGCCCTGC TAGGAAGTGG CGGAAGCCGT

1401    ACAAAGTGGC TGAAGCCCCC AGCAGAGAAC AAGGCCGAGG AGAACCGCTA

1451    TGTGGTCCGG CTAAGTGAGA GCAACCTGGT CATCTGAGGG GCGGTCTAGT

1501    CTAAGGACAC TGCGGCCCTG CCCTGGGAGG TTCCGAAGGC TTCCTGGAGG

1551    AGGTGGAGCT GCAGCTGGGA CTGTGAGGAC CGAGAAGCAA TGGCCCAGCA

1601    GACGAGACAG CAAAGACCAA GGCCTGGAGG TGGGAGCGTC TGCCCCAGTG

1651    AGGAGGCAGG TGGCCGGCGG GCACTGTGTA CAGGAGCAGG CTGAGCCCCG

1701    CCCCTGGCCC TGCTGCCATG TTGCTCCCCT GAAGGATGCC CCGACCCCCG

1751    TGCCTGCCCT GGCTGGATCC TAGGAGCCCA CGGGATTCTC TGTATCATCA

1801    GAGGCTGGGC TTGGCAGAGG GGAGGGGCCT GTGCCCGTCA CCCCTGGCCC

1851    CATTCCTTGG TAATTAGCCA CACCCTTGCC TCTGTACAGG GCCCTAGAGC

1901    AGATGTGCGT CCCCCTCCTC TTCCAGCAGG TCTATAAAGG GAAGGGGTAG

1951    CAGAAAGTCC TGGGCTAGGA GAGTGAGTCC CTGGGTTCTA ATCTTGGGCA

2001    CATCTGTGGC CATCGCTGGG TCCATTTTTC TGACTGTGAA GTAAGGAGAG

2051    ACGTCTCAGT ACCCAGGGCC TCTTCAGCTC TTTGTAGGTT CTGGGCTGGG

2101    TTGTGGGGGA CTGGGGAGCT GGGCTCTACC ATCCCTCCCA TTAGTAGCTT

2151    TATCCAGCCC CGTTTTTGCT GCTTCCAGGG CCTCTGCCTT CAAGGCCCCC

2201    ATGGGGCTGT CCATCCATGG CTCTGCCTAC GGAAGGGGCT TAATGCATGT
```

Figure 1 (cont)

```
2251   GCCTGCCCCT  CCCCCAGCTG  TTTTTAATGA  AACTGAAAAA  ATAGACTTGA

2301   TCCCGGCAGG  ACTGTGATAC  AGAGCCCTAG  CCTGCCCAGC  CAGCCCCAAG

2351   ATCTCAGGAG  CTTTAGGGAG  AAGACTTGGT  GGGGCTGGAG  CACACCTTGG

2401   GCCTCAGTGG  TTTCTGTGTC  CCTGTGGTGC  CAGTGCTTCT  GGGCAGTGCA

2451   GGCGGCTGCC  AGGCCCAGCC  CTGACTTCCA  CTCTGGCTCA  GCAACCTGGT

2501   TATTTATGTG  GGGCCGTGCA  GGCATGGGCC  CACTGCCTGT  CCATCCTGTT

2551   TCTCTTATTT  ATTGAAACTC  ACCATTGCCC  TATCCTTGTG  TCTCCACCCC

2601   CTTCCATGTG  TTGAATAATA  AAAGGTGGGA  AAGTGCTG
```

Figure 2
cDNA Sequence of Mouse TNFr/OPG-Like Nucleic Acid Molecule
(SEQ ID NO. 3)

```
   1  CAGGCTGCGC GGCCGGCCCC GAGCGCTCGC CTAGCGGGGC CCCGGCGCCG

  51  CGTCGGACGC TGAGCGAAGC TGGTGCTGCG GGCCAGGTCA ATGTCACTCC

 101  AGGGCCTGAT GATGAAGCGG ACCTTGCTGT GCTGGCCCCT GTCTTGCCTC

 151  TTTGTGCTGC TGCCCTGGCC TCTGGCCACT CCAACACCAA TAACTCCTTG

 201  GCTGTGTCCA CCTGGCAAAG AGCCTGACCC AGATCCAGGA CAGGGCACAT

 251  TATGCAGAAC TTGCCCCCCA GGAACCTTTT CAGCCTCATG GAACTCCTAT

 301  CCATGCCAGC CTCATTACCG ATGCAGCCTT CAAAAGAGGC TGGAGGCCCA

 351  GGCTGGCACA GCAACTCATG ATACAATGTG TGGAGACTGC CAGCATGGGT

 401  GGTTTGGGCC ACAGGGAGTT CCTCATGTTC CGTGTCAGCC ATGTTCCAAG

 451  GCACCTCCAA GTACTGGTGG CTGTGATGAG TCAGGGCGGC GGGGCCGGCG

 501  TGGCGTCGAA GTGGCAGCAG GTACCAGTAG CAACGGTGAA CCTCGGCAGC

 551  CCGGGAATGG CACTCGGGCA GGCGGTCCTG AGGAGACGGC TGCCCAGTAT

 601  GCAGTGATTG CCATCGTTCC TGTCTTTTGT CTCATGGGGC TTCTGGGCAT

 651  CCTGGTGTGC AACCTGCTCA AGCGGAAGGG CTACCATTGC ACAGCCCAAA

 701  AGGAAGTTGG GCCCAGCCCT GGTGGAGGAG CAGCGGGAT TAATCCTGCC

 751  TATAGGACTG AAGATGCCAA CGAGGACACC ATTGGAGTCC TGGTGCGCCT

 801  GATCACAGAG AAGAAAGAGA ATGCAGCGGC CCTGGAGGAG CTGTTGAAAG

 851  AATATCACAG CAAACAGCTG GTACAGACAA GTCACAGGCC TGTACCCAGG

 901  CTGCTGCCGG CCTCACCCAG CATACCCCAC ATCTGCCCGC ATCACCACCA

 951  CCTGCACACT GTGCAGGGCC TGGCCTCACT CTCTGGCCCC TGTTGCTCCC

1001  GTTGTAGCCA GAAGTGGCCA GAGGTGCTGC TGTCTCCTGA GGCAGCAGCT

1051  GCCACCACTC CTGCTCCCAC CCTTCTGCCT ACTGCATCCA GGGCTCCCAA
```

Figure 2 (cont)

```
1101   GGCTAGTGCC  AAGCCAGGAC  GTCAGGGCGA  GATTACCATC  TTGTCTGTGG

1151   GCAGGTTCCG  TGTGGCTCGT  ATTCCTGAGC  AGCGGACCAG  TTCATTGTTA

1201   TCTGAGGTGA  AGACCATCAC  GGAGGCTGGG  CCTTCAGAGG  GTGATCTCCC

1251   TGACTCCCCA  CAGCCTGGTT  TTCCCCCCGA  GCAGCGGGCA  CTGCTGGGAA

1301   GTGGTGGGAG  CCATACTAAG  TGGTTGAAGC  CCCCAGCAGA  GAACAAAGCT

1351   GAGGAGAACC  GCTATGTGGT  CCGGCTAAGT  GAAAGCAACC  TGGTCATCTG

1401   ATGGGCTGTC  TAGAATTAGA  CACTCTGCCC  TGTCCTGGGA  GGTTCTGAAG

1451   GCTTCCTGCA  GGAGGGAGAG  CTGCAGCTGG  GACTGAGGAC  CAAGATGCAA

1501   AGGCCAAGTC  CTGGAGGTGG  GACCGTCCGC  CCCACTGAGG  AGGCAGCCTG

1551   CGGCACAGCA  CGTGAGCAGG  AGATCAAGAG  CCCACCCTAT  CCCTGCAGTC

1601   CCGGTTACTT  CCATGCAGGG  TGCTGTAACC  CTGTGCCTGC  CCTGAACACA

1651   TCATAGGAGC  CCTCTGTCCC  TTAGAGGTCT  GGTTTGGTGG  AGGAGTGGTA

1701   TCTGTACCTG  GCCCCAAGCT  TGTGCCTTGG  GAACTAGCCA  CTCTTGCCCA

1751   TGTCCTGGAC  CCTGGATGTG  ACTCCCTCTC  TTCTGGCAGG  CCCTATAGAG

1801   GGAAGGGGTA  GCAAAGAGCC  CTGTACTGGT  GGCAGAGTAC  CTGGGTTCCA

1851   ATCCTGGGCT  TATCCCTAGG  TACGTAGGGG  AGGAGAACTC  AGTTCCCAGG

1901   ACCTCTCCAG  CTCTTTGCAG  ATTCTGGGCT  GAGTCCTGTT  GGGGGGAGCT

1951   TGACTTTGCT  ACCCTCCCAT  TAGTAGCTTT  ATCTGGCCTG  TTTTTGCTGC

2001   TTCCTGGGCC  TTGGCCTTCA  TGGCTCCCAT  GGGACTGTCT  ATTATGGTGA

2051   TGCCTTCAGA  AGGGGTTTAA  TGCATGTGCC  TGCCCCTACC  CTGCTATTTT

2101   TAATGAAACT  GAAAAATGAC  TTGACTTGGA  CAGGGCTCTC  TGGTGCAGAG

2151   CCTCAGTCCA  CCCTGCTGCC  CTCAAGCTCT  GGAGCTGTGG  AAGAGGAGA

2201   CAGGCAGGCT  AGGGAGTGCC  TGTGGCCTGT  GGTTTTCAAT  GCCCCTGTGG

2251   TACAGTATCT  GCCTGAGTTT  TGGGTAGCAG  GGGTGACTGC  CAATCCAGCC
```

Figure 2 (cont)
```
2301   TGTCTTAGTC TCTGCTCTGG CTCAGTGCCT CGTTATTTAT GTGGGGCCGT

2351   GCAGGCGCGG GGCCCACTGC CCATCCCATT TCTTATTTAT TGAAACCTGC

2401   TGTTGCCCTG CCCCTACATC TCCAGCCCCA CACACTTGAG TAATAAAAGG

2451   TGGAAAATGT CAAAAAAAA AAAAAAAGG
```

Figure 3

Predicted Amino acid sequence of Human TNFr/OPG-Like Polypeptide

(SEQ ID NO. 2)

```
  1   MKPSLLCRPL  SCFLMLLPWP  LATLTSTTLW  QCPPGEEPDL  DPGQGTLCRP

 51   CPPGTFSAAW  GSSPCQPHAR  CSLWRRLEAQ  VGMATRDTLC  GDCWPGWFGP

101   WGVPRVPCQP  CSWAPLGTHG  CDEWGRRARR  GVEVAAGASS  GGETRQPGNG

151   TRAGGPEETA  AQYAVIAIVP  VFCLMGLLGI  LVCNLLKRKG  YHCTAHKEVG

201   PGPGGGGSGI  NPAYRTEDAN  EDTIGVLVRL  ITEKKENAAA  LEELLKEYHS

251   KQLVQTSHRP  VSKLPPAPPN  VPHICPHRHH  LHTVQGLASL  SGPCCSRCSQ

301   KKWPEVLLSP  EAVAATTPVP  SLLPNPTRVP  KAGAKAGRQG  EITILSVGRF

351   RVARIPEQRT  SSMVSEVKTI  TEAGPSWGDL  PDSPQPGLPP  EQQALLGSGG

401   SRTKWLKPPA  ENKAEENRYV  VRLSESNLVI  *
```

Figure 4

Predicted Amino acid sequence of Mouse TNFr/OPG-Like
Polypeptide

(SEQ ID NO:4)

```
  1   MSLQGLMMKR  TLLCWPLSCL  FVLLPWPLAT  PTPITPWLCP  PGKEPDPDPG

 51   QGTLCRTCPP  GTFSASWNSY  PCQPHYRCSL  QKRLEAQAGT  ATHDTMCGDC

101   QHGWFGPQGV  PHVPCQPCSK  APPSTGGCDE  SGRRGRRGVE  VAAGTSSNGE

151   PRQPGNGTRA  GGPEETAAQY  AVIAIVPVFC  LMGLLGILVC  NLLKRKGYHC

201   TAQKEVGPSP  GGGGSGINPA  YRTEDANEDT  IGVLVRLITE  KKENAAALEE

251   LLKEYHSKQL  VQTSHRPVPR  LLPASPSIPH  ICPHHHHLHT  VQGLASLSGP

301   CCSRCSQKWP  EVLLSPEAAA  ATTPAPTLLP  TASRAPKASA  KPGRQGEITI

351   LSVGRFRVAR  IPEQRTSSLL  SEVKTITEAG  PSEGDLPDSP  QPGFPPEQRA

401   LLGSGGSHTK  WLKPPAENKA  EENRYVVRLS  ESNLVI*
```

Figure 5

Human TNFr/OPG-Like cDNA with Predicted Amino Acid Sequence

(SEQ ID NO: 1 and SEQ ID NO: 2)

```
  5        1 CGGGACCTTCAGATATCCCCTCCCAGCCGAGGGGGCTTCCATCTAACTGTTTTTTTGGTC 60
              G   P   S   D   I   P   S   Q   P   R   G   L   P   S   N   C   F   F   G   H  -

           61 ACGGTTCCAGGGCCGTTTTAGACAGTGGAGGCCTTGTGGGGCAGGGTGTGAGGGGTGCTG 120
 10           G   S   R   A   V   L   D   S   G   G   L   V   G   Q   G   V   R   G   A   E  -

          121 AGCAGCAGGTGTGGACATGTGTGTGCACCAGGCCTTTCTACCTGACCGGGCCGGCGACCA 180
              Q   Q   V   W   T   C   V   C   T   R   P   F   Y   L   T   G   P   A   T   T  -

 15       181 CCAGGGGCCTGAGGATGAAGCCAAGTCTGCTGTGCCGGCCCCTGTCCTGCTTCCTTATGC 240
              R   G   L   R   M   K   P   S   L   L   C   R   P   L   S   C   F   L   M   L  -

          241 TGCTGCCCTGGCCTCTCGCCACCCTGACATCAACAACCCTTTGGCAGTGCCCACCTGGGG 300
              L   P   W   P   L   A   T   L   T   S   T   T   L   W   Q   C   P   P   G   E  -

 20       301 AGGAGCCCGACCTGGACCCAGGGCAGGGCACATTATGCAGGCCCTGCCCCCCAGGCACCT 360
              E   P   D   L   D   P   G   Q   G   T   L   C   R   P   C   P   P   G   T   F  -

          361 TCTCAGCTGCATGGGGCTCCAGCCCATGCCAGCCCCATGCCCGTTGCAGCCTTTGGAGGA 420
 25           S   A   A   W   G   S   S   P   C   Q   P   H   A   R   C   S   L   W   R   R  -

          421 GGCTGGAGGCCCAGGTGGGCATGGCAACTCGAGATACACTCTGTGGAGACTGCTGGCCTG 480
              L   E   A   Q   V   G   M   A   T   R   D   T   L   C   G   D   C   W   P   G  -

 30       481 GGTGGTTTGGGCCTTGGGGGGGTTCCCCGCGTTCCATGTCAACCATGTTCCTGGGCACCTC 540
              W   F   G   P   W   G   V   P   R   V   P   C   Q   P   C   S   W   A   P   L  -

          541 TGGGTACTCATGGCTGTGATGAGTGGGGGCGGCGGGCCCGACGTGGCGTGGAGGTGGCAG 600
              G   T   H   G   C   D   E   W   G   R   R   A   R   R   G   V   E   V   A   A  -

 35       601 CAGGGGCCAGCAGCGGTGGTGAGACACGGCAGCCTGGGAACGGCACCCGGGCAGGTGGCC 660
              G   A   S   S   G   G   E   T   R   Q   P   G   N   G   T   R   A   G   G   P  -

          661 CAGAGGAGACAGCCGCCCAGTACGCGGTCATCGCCATCGTCCCTGTCTTCTGCCTCATGG 720
              E   E   T   A   A   Q   Y   A   V   I   A   I   V   P   V   F   C   L   M   G  -

 40       721 GGCTGTTGGGCATCCTGGTGTGCAACCTCCTCAAGCGGAAGGGCTACCACTGCACGGCGC 780
              L   L   G   I   L   V   C   N   L   L   K   R   K   G   Y   H   C   T   A   H  -

          781 ACAAGGAGGTCGGGCCCGGCCCTGGAGGTGGAGGCAGTGGAATCAACCCTGCCTACCGGA 840
 45           K   E   V   G   P   G   P   G   G   G   G   S   G   I   N   P   A   Y   R   T  -

          841 CTGAGGATGCCAATGAGGACACCATTGGGGTCCTGGTGCGCTTGATCACAGAGAAGAAAG 900
              E   D   A   N   E   D   T   I   G   V   L   V   R   L   I   T   E   K   K   E  -

 50       910 AGAATGCTGCGGCCCTGGAGGAGCTGCTGAAAGAGTACCACAGCAAACAGCTGGTGCAGA 960
              N   A   A   A   L   E   E   L   L   K   E   Y   H   S   K   Q   L   V   Q   T  -

          961 CGAGCCACAGGCCTGTGTCCAAGCTGCCGCCAGCGCCCCCGAACGTGCCACACATCTGCC 1020
 55           S   H   R   P   V   S   K   L   P   P   A   P   P   N   V   P   H   I   C   P  -

         1021 CGCACCGCCACCATCTCCACACCGTGCAGGGCCTGGCCTCGCTCTCTGGCCCCTGCTGCT 1080
              H   R   H   H   L   H   T   V   Q   G   L   A   S   L   S   G   P   C   C   S  -
```

Figure 5 (cont.)

```
1081 CCCGCTGTAGCCAGAAGAAGTGGCCCGAGGTGCTGCTGTCCCCTGAGGCTGTAGCCGCCA 1140
      R  C  S  Q  K  K  W  P  E  V  L  L  S  P  E  A  V  A  A  T -

1141 CTACTCCTGTTCCCAGCCTTCTGCCTAACCCGACCAGGGTTCCCAAGGCCGGGGCCAAGG 1200
      T  P  V  P  S  L  L  P  N  P  T  R  V  P  K  A  G  A  K  A -

1201 CAGGGCGTCAGGGCGAGATCACCATCTTGTCTGTGGGCAGGTTCCGCGTGGCTCGAATTC 1260
      G  R  Q  G  E  I  T  I  L  S  V  G  R  F  R  V  A  R  I  P -

1261 CTGAGCAGCGGACAAGTTCAATGGTGTCTGAGGTGAAGACCATCACGGAGGCTGGGCCCT 1320
      E  Q  R  T  S  S  M  V  S  E  V  K  T  I  T  E  A  G  P  S -

1321 CGTGGGGTGATCTCCCTGACTCCCCACAGCCTGGCCTCCCCCCTGAGCAGCAGGCCCTGC 1380
      W  G  D  L  P  D  S  P  Q  P  G  L  P  P  E  Q  Q  A  L  L -

1381 TAGGAAGTGGCGGAAGCCGTACAAAGTGGCTGAAGCCCCCAGCAGAGAACAAGGCCGAGG 1440
      G  S  G  G  S  R  T  K  W  L  K  P  P  A  E  N  K  A  E  E -

1441 AGAACCGCTATGTGGTCCGGCTAAGTGAGAGCAACCTGGTCATCTGAGGGGCGGTCTAGT 1500
      N  R  Y  V  V  R  L  S  E  S  N  L  V  I  *  G  A  V  *  S -

1501 CTAAGGACACTGCGGCCCTGCCCTGGGAGGTTCCGAAGGCTTCCTGGAGGAGGTGGAGCT 1560
      K  D  T  A  A  L  P  W  E  V  P  K  A  S  W  R  R  W  S  C -

1561 GCAGCTGGGACTGTGAGGACCGAGAAGCAATGGCCCAGCAGACGAGACAGCAAAGACCAA 1620
      S  W  D  C  E  D  R  E  A  M  A  Q  Q  T  R  Q  Q  R  P  R -

1621 GGCCTGGAGGTGGGAGCGTCTGCCCCAGTGAGGAGGCAGGTGGCCGGCGGGCACTGTGTA 1680
      P  G  G  S  V  C  P  S  E  E  A  G  G  R  R  A  L  C  T -

1681 CAGGAGCAGGCTGAGCCCCGCCCCTGGCCCTGCTGCCATGTTGCTCCCCTGAAGGATGCC 1740
      G  A  G  *  A  P  P  L  A  L  L  F  C  C  S  P  E  G  C  P -

1741 CCGACCCCCGTGCCTGCCCTGGCTGGATCCTAGGAGCCCACGGGATTCTCTGTATCATCA 1800
      D  P  P  A  C  P  G  W  I  L  G  A  H  G  I  L  C  I  I  R -

1801 GAGGCTGGGCTTGGCAGAGGGGAGGGGCCTGTGCCCGTCACCCCTGGCCCCATTCCTTGG 1860
      G  W  A  W  Q  R  G  G  A  C  A  R  H  P  W  P  H  S  L  V -

1861 TAATTAGCCACACCCTTGCCTCTGTACAGGGCCCTAGAGCAGATGTGCGTCCCCCTCCTC 1920
      I  S  H  T  L  A  S  V  Q  G  P  R  A  D  V  R  P  P  P  L -

1921 TTCCAGCAGGTCTATAAAGGGAAGGGGTAGCAGAAAGTCCTGGGCTAGGAGAGTGAGTCC 1980
      P  A  G  L  *  R  E  G  V  A  E  S  P  G  L  G  E  *  V  P -

1981 CTGGGTTCTAATCTTGGGCACATCTGTGGCCATCGCTGGGTCCATTTTTCTGACTGTGAA 2040
      G  F  *  S  W  A  H  L  W  P  S  L  G  P  F  F  *  L  *  S -

2041 GTAAGGAGAGACGTCTCAGTACCCAGGGCCTCTTCAGCTCTTTGTAGGTTCTGGGCTGGG 2100
      K  E  R  R  L  S  T  Q  G  L  F  S  S  L  *  V  L  G  W  V -

2101 TTGTGGGGGACTGGGGAGCTGGGCTCTACCATCCCTCCCATTAGTAGCTTTATCCAGCCC 2160
      V  G  D  W  G  A  G  L  Y  H  P  S  H  *  *  L  Y  P  A  P -

2161 CGTTTTGCTGCTTCCAGGGCCTCTGCCTTCAAGGCCCCCATGGGGCTGTCCATCCATGG 2220
      F  L  L  L  P  G  P  L  P  S  R  F  P  W  G  C  P  S  M  A -
```

Figure 5 (cont.)

```
2221 CTCTGCCTACGGAAGGGGCTTAATGCATGTGCCTGCCCCTCCCCCAGCTGTTTTTAATGA 2280
      L  P  T  E  G  A  *  C  M  C  L  P  L  P  Q  L  F  L  M  K -

2281 AACTGAAAAAATAGACTTGATCCCGGCAGGACTGTGATACAGAGCCCTAGCCTGCCCAGC 2340
      L  K  K  *  T  *  S  R  Q  D  C  D  T  E  P  *  P  A  Q  P -

2341 CAGCCCCAAGATCTCAGGAGCTTTAGGGAGAAGACTTGGTGGGGCTGGAGCACACCTTGG 2400
      A  P  R  S  Q  E  L  *  G  E  D  L  V  G  L  E  H  T  L  G -

2401 GCCTCAGTGGTTTCTGTGTCCCTGTGGTGCCAGTGCTTCTGGGCAGTGCAGGCGGCTGCC 2460
      L  S  G  F  C  V  P  V  V  P  V  L  L  G  S  A  G  G  C  Q -

2461 AGGCCCAGCCCTGACTTCCACTCTGGCTCAGCAACCTGGTTATTTATGTGGGGCCGTGCA 2520
      A  Q  P  *  L  P  L  W  L  S  N  L  V  I  Y  V  G  P  C  R -

2521 GGCATGGGCCCACTGCCTGTCCATCCTGTTTCTCTTATTTATTGAAACTCACCATTGCCC 2580
      H  G  P  T  A  C  P  S  C  F  S  Y  L  L  K  L  T  I  A  L -

2581 TATCCTTGTGTCTCCACCCCCTTCCATGTGTTGAATAATAAAAGGTGGGAAAGTGCTG   2638
      S  L  C  L  H  P  L  P  C  V  E  *  *  K  V  G  K  C     -
```

Figure 6

Mouse TNFr/OPG-Like cDNA with Predicted Amino Acid Sequence

(SEQ ID NO: 3 and SEQ ID NO: 4)

```
   1 CAGGCTGCGCGGCCGGCCCCGAGCGCTCGCCTAGCGGGGCCCCGGCGCCGCGTCGGACGC  60


  61 TGAGCGAAGCTGGTGCTGCGGGCCAGGTCAATGTCACTCCAGGGCCTGATGATGAAGCGG 120
                              M   S   L   Q   G   L   M   M   K   R   -

 121 ACCTTGCTGTGCTGGCCCCTGTCTTGCCTCTTTGTGCTGCTGCCCTGGCCTCTGGCCACT 180
      T   L   L   C   W   P   L   S   C   L   F   V   L   L   P   W   P   L   A   T   -

 181 CCAACACCAATAACTCCTTGGCTGTGTCCACCTGGCAAAGAGCCTGACCCAGATCCAGGA 240
      P   T   P   I   T   P   W   L   C   P   P   G   K   E   P   D   P   D   P   G   -

 241 CAGGGCACATTATGCAGAACTTGCCCCCCAGGAACCTTTTCAGCCTCATGGAACTCCTAT 300
      Q   G   T   L   C   R   T   C   P   P   G   T   F   S   A   S   W   N   S   Y   -

 301 CCATGCCAGCCTCATTACCGATGCAGCCTTCAAAAGAGGCTGGAGGCCCAGGCTGGCACA 360
      P   C   Q   P   H   Y   R   C   S   L   Q   K   R   L   E   A   Q   A   G   T   -

 361 GCAACTCATGATACAATGTGTGGAGACTGCCAGCATGGGTGGTTTGGGCCACAGGGAGTT 420
      A   T   H   D   T   M   C   G   D   C   Q   H   G   W   F   G   P   Q   G   V   -

 421 CCTCATGTTCCGTGTCAGCCATGTTCCAAGGCACCTCCAAGTACTGGTGGCTGTGATGAG 480
      P   H   V   P   C   Q   P   C   S   K   A   P   P   S   T   G   G   C   D   E   -

 481 TCAGGGCGGCGGGGCCGGCGTGGCGTCGAAGTGGCAGCAGGTACCAGTAGCAACGGTGAA 540
      S   G   R   R   G   R   R   G   V   E   V   A   A   G   T   S   S   N   G   E   -

 541 CCTCGGCAGCCCGGGAATGGCACTCGGGCAGGCGGTCCTGAGGAGACGGCTGCCCAGTAT 600
      P   R   Q   P   G   N   G   T   R   A   G   G   P   E   E   T   A   A   Q   Y   -

 601 GCAGTGATTGCCATCGTTCCTGTCTTTTGTCTCATGGGGCTTCTGGGCATCCTGGTGTGC 660
      A   V   I   A   I   V   P   V   F   C   L   M   G   L   L   G   I   L   V   C   -

 661 AACCTGCTCAAGCGGAAGGGCTACCATTGCACAGCCCAAAAGGAAGTTGGGCCCAGCCCT 720
      N   L   L   K   R   K   G   Y   H   C   T   A   Q   K   E   V   G   P   S   P   -

 721 GGTGGAGGAGGCAGCGGGATTAATCCTGCCTATAGGACTGAAGATGCCAACGAGGACACC 780
      G   G   G   G   S   G   I   N   P   A   Y   R   T   E   D   A   N   E   D   T   -

 781 ATTGGAGTCCTGGTGCGCCTGATCACAGAGAAGAAAGAGAATGCAGCGGCCCTGGAGGAG 840
      I   G   V   L   V   R   L   I   T   E   K   K   E   N   A   A   A   L   E   E   -

 841 CTGTTGAAAGAATATCACAGCAAACAGCTGGTACAGACAAGTCACAGGCCTGTACCCAGG 900
      L   L   K   E   Y   H   S   K   Q   L   V   Q   T   S   H   R   P   V   P   R   -

 901 CTGCTGCCGGCCTCACCCAGCATACCCCACATCTGCCCGCATCACCACCACCTGCACACT 960
      L   L   P   A   S   P   S   I   P   H   I   C   P   H   H   H   H   L   H   T   -

 961 GTGCAGGGCCTGGCCTCACTCTCTGGCCCCTGTTGCTCCCGTTGTAGCCAGAAGTGGCCA 1020
      V   Q   G   L   A   S   L   S   G   P   C   C   S   R   C   S   Q   K   W   P   -

1021 GAGGTGCTGCTGTCTCCTGAGGCAGCAGCTGCCACCACTCCTGCTCCCACCCTTCTGCCT 1080
      E   V   L   L   S   P   E   A   A   A   A   T   T   P   A   P   T   L   L   P   -
```

Figure 6 (cont.)

```
1081 ACTGCATCCAGGGCTCCCAAGGCTAGTGCCAAGCCAGGACGTCAGGGCGAGATTACCATC 1140
      T  A  S  R  A  P  K  A  S  A  K  P  G  R  Q  G  E  I  T  I  -

1141 TTGTCTGTGGGCAGGTTCCGTGTGGCTCGTATTCCTGAGCAGCGGACCAGTTCATTGTTA 1200
      L  S  V  G  R  F  R  V  A  R  I  P  E  Q  R  T  S  S  L  L  -

1201 TCTGAGGTGAAGACCATCACGGAGGCTGGGCCTTCAGAGGGTGATCTCCCTGACTCCCCA 1260
      S  E  V  K  T  I  T  E  A  G  P  S  E  G  D  L  P  D  S  P  -

1261 CAGCCTGGTTTTCCCCCCGAGCAGCGGGCACTGCTGGGAAGTGGTGGGAGCCATACTAAG 1320
      Q  P  G  F  P  P  E  Q  R  A  L  L  G  S  G  G  S  H  T  K  -

1321 TGGTTGAAGCCCCCAGCAGAGAACAAAGCTGAGGAGAACCGCTATGTGGTCCGGCTAAGT 1380
      W  L  K  P  P  A  E  N  K  A  E  E  N  R  Y  V  V  R  L  S  -

1381 GAAAGCAACCTGGTCATCTGATGGGCTGTCTAGAATTAGACACTCTGCCCTGTCCTGGGA 1440
      E  S  N  L  V  I

1441 GGTTCTGAAGGCTTCCTGCAGGAGGGAGAGCTGCAGCTGGGACTGAGGACCAAGATGCAA 1500

1501 AGGCCAAGTCCTGGAGGTGGGACCGTCCGCCCCACTGAGGAGGCAGCCTGCGGCACAGCA 1560

1561 CGTGAGCAGGAGATCAAGAGCCCACCCTATCCCTGCAGTCCCGGTTACTTCCATGCAGGG 1620

1621 TGCTGTAACCCTGTGCCTGCCCTGAACACATCATAGGAGCCCTCTGTCCCTTAGAGGTCT 1680

1681 GGTTTGGTGGAGGAGTGGTATCTGTACCTGGCCCCAAGCTTGTGCCTTGGGAACTAGCCA 1740

1741 CTCTTGCCCATGTCCTGGACCCTGGATGTGACTCCCTCTCTTCTGGCAGGCCCTATAGAG 1800

1801 GGAAGGGGTAGCAAAGAGCCCTGTACTGGTGGCAGAGTACCTGGGTTCCAATCCTGGGCT 1860

1861 TATCCCTAGGTACGTAGGGGAGGAGAACTCAGTTCCCAGGACCTCTCCAGCTCTTTGCAG 1920

1921 ATTCTGGGCTGAGTCCTGTTGGGGGGAGCTTGACTTTGCTACCCTCCCATTAGTAGCTTT 1980

1981 ATCTGGCCTGTTTTTGCTGCTTCCTGGGCCTTGGCCTTCATGGCTCCCATGGGACTGTCT 2040

2041 ATTATGGTGATGCCTTCAGAAGGGGTTTAATGCATGTGCCTGCCCCTACCCTGCTATTTT 2100

2101 TAATGAAACTGAAAAATGACTTGACTTGGACAGGGCTCTCTGGTGCAGAGCCTCAGTCCA 2160

2161 CCCTGCTGCCCTCAAGCTCTGGAGCTGTGGGAAGAGGAGACAGGCAGGCTAGGGAGTGCC 2220
```

Figure 6 (cont.)

2221 TGTGGCCTGTGGTTTTCAATGCCCCTGTGGTACAGTATCTGCCTGAGTTTTGGGTAGCAG 2280

2281 GGGTGACTGCCAATCCAGCCTGTCTTAGTCTCTGCTCTGGCTCAGTGCCTCGTTATTTAT 2340

2341 GTGGGGCCGTGCAGGCGCGGGGCCCACTGCCCATCCCATTTCTTATTTATTGAAACCTGC 2400

2401 TGTTGCCCTGCCCCTACATCTCCAGCCCCACACACTTGAGTAATAAAAGGTGGAAAATGT 2460

2461 CAAAAAAAAAAAAAAAAGG   2479

Figure 7

(SEQ ID NO: 5 and SEQ ID NO: 6)

```
    CTGTCCTGGGAGGGCCCTGAGGGCCAGGGGCAGAGTCCTGTGCCTGGCCCCCAAGGGTCC
5

    TCAGGCTTGGCTCCTGGCCATGCTCTCACCCTTTACCTCCCACAGGACCCAGGGCAGGGC
a                                                   D   P   G   Q   G   -

    ACATTATGCAGGCCCTGCCCCCCAGGCACCTTCTCAGCTGCATGGGGCTCCAGCCCATGC
10  a     T   L   C   R   P   C   P   P   G   T   F   S   A   A   W   G   S   S   P   C   -

    CAGCCCCATGCCCGTTGCAGCCTTTGGAGGAGGCTGGAGGCCCAGGTGGGCATGGCAACT
a     Q   P   H   A   R   C   S   L   W   R   R   L   E   A   Q   V   G   M   A   T   -

15  CGAGATACACTCTGTGGAGACTGCTGGCCTGGGTAAGCCAAAGGGAGTGCGGGGAGGGCT
a     R   D   T   L   C   G   D   C   W   P   G                               -

    CCTGGCTGGGTGACCAGGACTCTGGATCCTGGGGCCCCAGCCTTATTGTACCCTGAGCAG

20  GCCTCATTCTTCCCATCTGTGAAATGGGATGGGGCAGGACCACGGAGGGTGCCTGGTAGG

    AAGGAATCCAGCCTCTCCTAAGGATAGTGTTTGGGGAAACTTCTGGGCCTCAGTGGTATC

    TT
25
```

FIGURE 8

Peptide Sequence Comparison of Human Osteoprotegerin (OPG) with TNFr/OPG-Like

Polypeptide

(SEQ ID NO: 7 and SEQ ID NO:8)

5

```
                  28        58        88        118       148
TNFr/OPG-Like    LSWEGPEGQGQSPVPGPQGSSGLAPGHALTLYLPQDPGQGTLCRPCPPGTFS-AAWG
                                    :: :   :   |  :  |:|:  ||  |  ||   : :
OPG              CNRTHNRVCECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSS
                 100       110       120       130       140       150
```

```
                  178       208       238       268
TNFr/OPG-Like    SSPCQPHARCSLWRRLEAQVGMATRDTLCGDCWPG
                 :||: |: ||:    | :| | ||:| :|::
OPG              KAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFT
                 160       170       180       190       200       210
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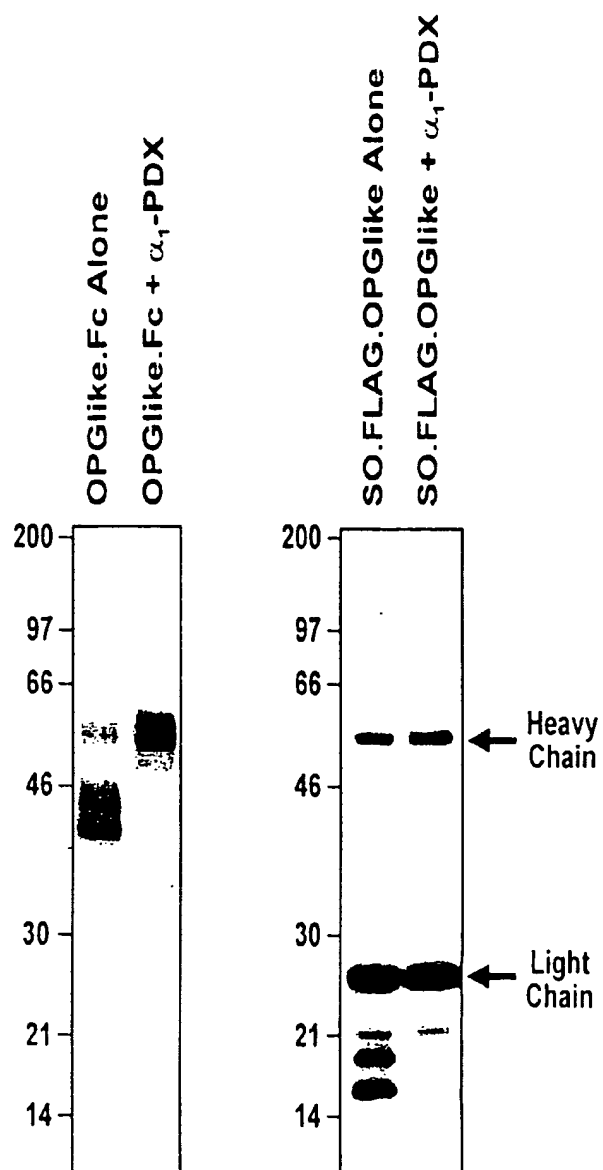
Figure 9

Figure 10
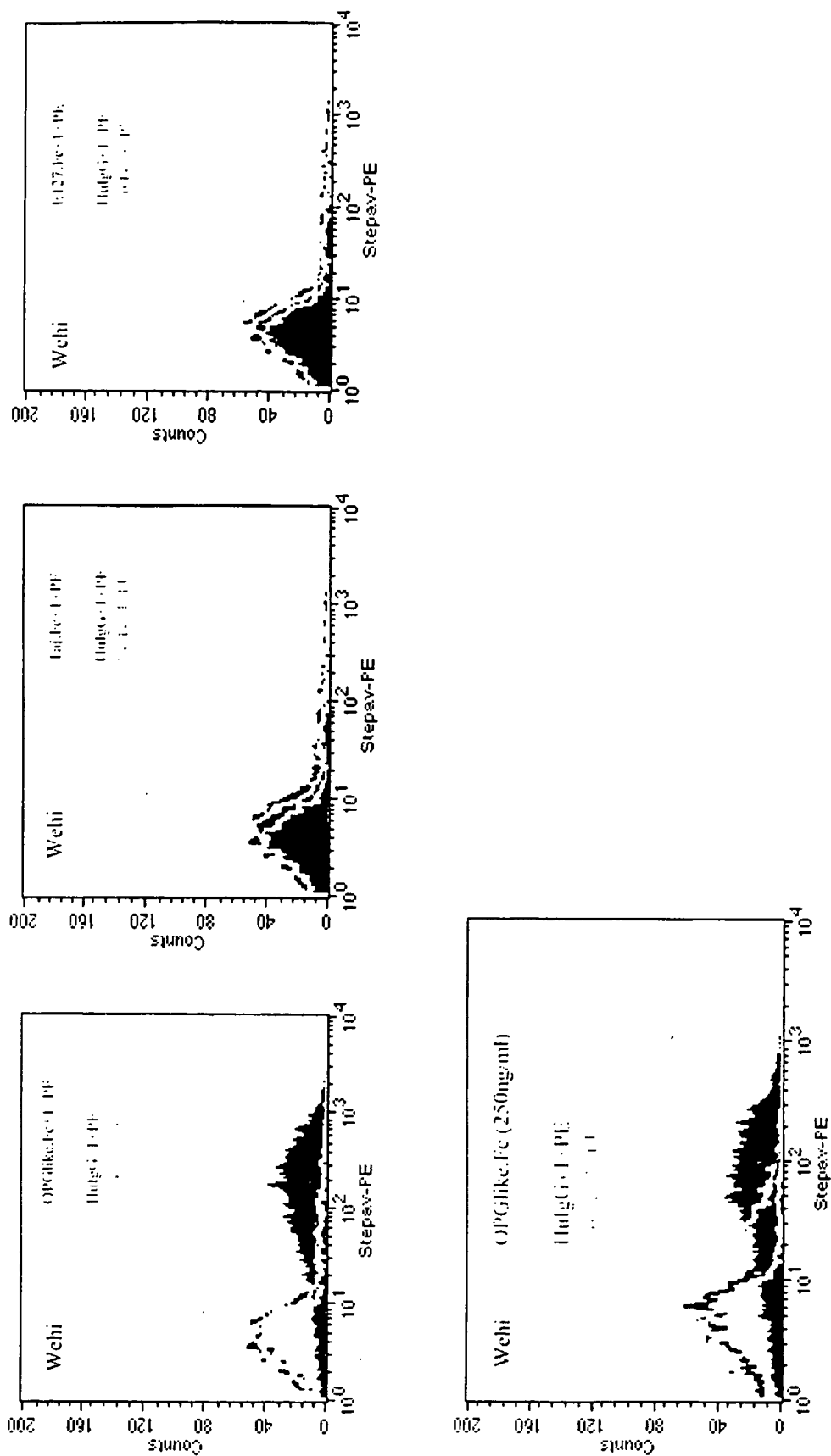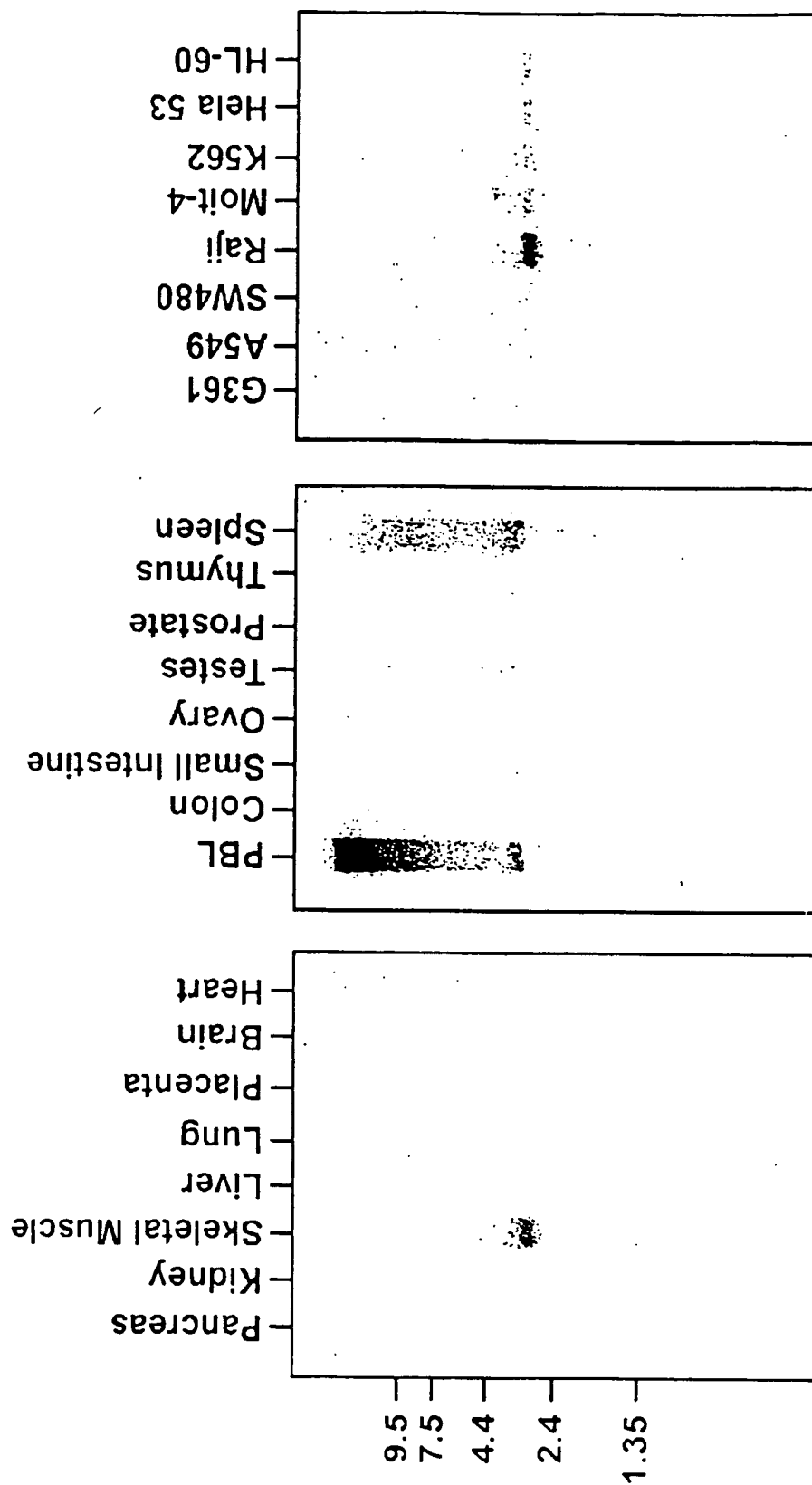
Figure 11

- 1 -

SEQUENCE LISTING

<110> AMGEN, INC.
     Jing, Shuqian (US only)
     Welcher, Andrew A (US only)
     Boedigheimer, Michael J (US only)
     Shu, Junyan (US only)
   Gary M. Fox (US only)

<120> TNFr/OPG-LIKE MOLECULES AND USES THEREOF

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<150> 60/172,306
<151> 1999-12-16

<160> 28

<170> PatentIn Ver. 2.0

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agcagcaggt gtggacatgt gtgtgcacca ggcctttcta cctgaccggg ccggcgacca 180

ccaggggcct gagg atg aag cca agt ctg ctg tgc cgg ccc ctg tcc tgc   230
              Met Lys Pro Ser Leu Leu Cys Arg Pro Leu Ser Cys
               1   5   10

ttc ctt atg ctg ctg ccc tgg cct ctc gcc acc ctg aca tca aca acc   278
Phe Leu Met Leu Leu Pro Trp Pro Leu Ala Thr Leu Thr Ser Thr Thr
           15   20   25

ctt tgg cag tgc cca cct ggg gag gag ccc gac ctg gac cca ggg cag   326
Leu Trp Gln Cys Pro Pro Gly Glu Glu Pro Asp Leu Asp Pro Gly Gln
       30   35   40

ggc aca tta tgc agg ccc tgc ccc cca ggc acc ttc tca gct gca tgg   374
Gly Thr Leu Cys Arg Pro Cys Pro Pro Gly Thr Phe Ser Ala Ala Trp
   45   50   55   60

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ggc tcc agc cca tgc cag ccc cat gcc cgt tgc agc ctt tgg agg agg    422
Gly Ser Ser Pro Cys Gln Pro His Ala Arg Cys Ser Leu Trp Arg Arg
                 65              70                  75

ctg gag gcc cag gtg ggc atg gca act cga gat aca ctc tgt gga gac    470
Leu Glu Ala Gln Val Gly Met Ala Thr Arg Asp Thr Leu Cys Gly Asp
             80              85                  90

tgc tgg cct ggg tgg ttt ggg cct tgg ggg gtt ccc cgc gtt cca tgt    518
Cys Trp Pro Gly Trp Phe Gly Pro Trp Gly Val Pro Arg Val Pro Cys
             95              100                 105

caa cca tgt tcc tgg gca cct ctg ggt act cat ggc tgt gat gag tgg    566
Gln Pro Cys Ser Trp Ala Pro Leu Gly Thr His Gly Cys Asp Glu Trp
    110                 115                 120

ggg cgg cgg gcc cga cgt ggc gtg gag gtg gca gca ggg gcc agc agc    614
Gly Arg Arg Ala Arg Arg Gly Val Glu Val Ala Ala Gly Ala Ser Ser
125                 130                 135                 140

ggt ggt gag aca cgg cag cct ggg aac ggc acc cgg gca ggt ggc cca    662
Gly Gly Glu Thr Arg Gln Pro Gly Asn Gly Thr Arg Ala Gly Gly Pro
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gag gag aca gcc gcc cag tac gcg gtc atc gcc atc gtc cct gtc ttc    710
Glu Glu Thr Ala Ala Gln Tyr Ala Val Ile Ala Ile Val Pro Val Phe
             160                 165                 170

tgc ctc atg ggg ctg ttg ggc atc ctg gtg tgc aac ctc ctc aag cgg    758
Cys Leu Met Gly Leu Leu Gly Ile Leu Val Cys Asn Leu Leu Lys Arg
             175                 180                 185

aag ggc tac cac tgc acg gcg cac aag gag gtc ggg ccc ggc cct gga    806
Lys Gly Tyr His Cys Thr Ala His Lys Glu Val Gly Pro Gly Pro Gly
        190                 195                 200

ggt gga ggc agt gga atc aac cct gcc tac cgg act gag gat gcc aat    854
Gly Gly Gly Ser Gly Ile Asn Pro Ala Tyr Arg Thr Glu Asp Ala Asn
205                 210                 215                 220

gag gac acc att ggg gtc ctg gtg cgc ttg atc aca gag aag aaa gag    902
Glu Asp Thr Ile Gly Val Leu Val Arg Leu Ile Thr Glu Lys Lys Glu
                 225                 230                 235

aat gct gcg gcc ctg gag gag ctg ctg aaa gag tac cac agc aaa cag    950
Asn Ala Ala Ala Leu Glu Glu Leu Leu Lys Glu Tyr His Ser Lys Gln
                 240                 245                 250

ctg gtg cag acg agc cac agg cct gtg tcc aag ctg ccg cca gcg ccc    998
Leu Val Gln Thr Ser His Arg Pro Val Ser Lys Leu Pro Pro Ala Pro
                 255                 260                 265
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ccg aac gtg cca cac atc tgc ccg cac cgc cac cat ctc cac acc gtg    1046
Pro Asn Val Pro His Ile Cys Pro His Arg His His Leu His Thr Val
    270             275             280

cag ggc ctg gcc tcg ctc tct ggc ccc tgc tgc tcc cgc tgt agc cag    1094
Gln Gly Leu Ala Ser Leu Ser Gly Pro Cys Cys Ser Arg Cys Ser Gln
285             290             295             300

aag aag tgg ccc gag gtg ctg ctg tcc cct gag gct gta gcc gcc act    1142
Lys Lys Trp Pro Glu Val Leu Leu Ser Pro Glu Ala Val Ala Ala Thr
            305             310             315

act cct gtt ccc agc ctt ctg cct aac ccg acc agg gtt ccc aag gcc    1190
Thr Pro Val Pro Ser Leu Leu Pro Asn Pro Thr Arg Val Pro Lys Ala
            320             325             330

ggg gcc aag gca ggg cgt cag ggc gag atc acc atc ttg tct gtg ggc    1238
Gly Ala Lys Ala Gly Arg Gln Gly Glu Ile Thr Ile Leu Ser Val Gly
            335             340             345

agg ttc cgc gtg gct cga att cct gag cag cgg aca agt tca atg gtg    1286
Arg Phe Arg Val Ala Arg Ile Pro Glu Gln Arg Thr Ser Ser Met Val
    350             355             360

tct gag gtg aag acc atc acg gag gct ggg ccc tcg tgg ggt gat ctc    1334
Ser Glu Val Lys Thr Ile Thr Glu Ala Gly Pro Ser Trp Gly Asp Leu
365             370             375             380

cct gac tcc cca cag cct ggc ctc ccc cct gag cag cag gcc ctg cta    1382
Pro Asp Ser Pro Gln Pro Gly Leu Pro Pro Glu Gln Gln Ala Leu Leu
            385             390             395

gga agt ggc gga agc cgt aca aag tgg ctg aag ccc cca gca gag aac    1430
Gly Ser Gly Gly Ser Arg Thr Lys Trp Leu Lys Pro Pro Ala Glu Asn
            400             405             410

aag gcc gag gag aac cgc tat gtg gtc cgg cta agt gag agc aac ctg    1478
Lys Ala Glu Glu Asn Arg Tyr Val Val Arg Leu Ser Glu Ser Asn Leu
            415             420             425

gtc atc tgaggggcgg tctagtctaa ggacactgcg ccctgccct gggaggttcc    1534
Val Ile
    430


gaaggcttcc tggaggaggt ggagctgcag ctgggactgt gaggaccgag aagcaatggc 1594

ccagcagacg agacagcaaa gaccaaggcc tggaggtggg agcgtctgcc ccagtgagga 1654

ggcaggtggc cggcgggcac tgtgtacagg agcaggctga gccccgcccc tggccctgct 1714

gccatgttgc tcccctgaag gatgccccga ccccgtgcc tgccctggct ggatcctagg 1774

agcccacggg attctctgta tcatcagagg ctgggcttgg cagaggggag gggcctgtgc 1834

ccgtcacccc tggccccatt ccttggtaat tagccacacc cttgcctctg tacagggccc 1894
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tagagcagat gtgcgtcccc ctcctcttcc agcaggtcta taaagggaag gggtagcaga 1954

aagtcctggg ctaggagagt gagtccctgg gttctaatct tgggcacatc tgtggccatc 2014

gctgggtcca tttttctgac tgtgaagtaa ggagagacgt ctcagtaccc agggcctctt 2074

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gcccccatgg ggctgtccat ccatggctct gcctacggaa ggggcttaat gcatgtgcct 2254

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Pro Pro Gly Glu Glu Pro Asp Leu Asp Pro Gly Gln Gly Thr Leu Cys
            35   40   45

Arg Pro Cys Pro Pro Gly Thr Phe Ser Ala Ala Trp Gly Ser Ser Pro
    50   55   60

Cys Gln Pro His Ala Arg Cys Ser Leu Trp Arg Arg Leu Glu Ala Gln
65   70   75   80

Val Gly Met Ala Thr Arg Asp Thr Leu Cys Gly Asp Cys Trp Pro Gly
                85   90   95

Trp Phe Gly Pro Trp Gly Val Pro Arg Val Pro Cys Gln Pro Cys Ser
            100   105   110

Trp Ala Pro Leu Gly Thr His Gly Cys Asp Glu Trp Gly Arg Arg Ala
        115   120   125

Arg Arg Gly Val Glu Val Ala Ala Gly Ala Ser Ser Gly Gly Glu Thr
    130   135   140

Arg Gln Pro Gly Asn Gly Thr Arg Ala Gly Gly Pro Glu Glu Thr Ala
145   150   155   160

Ala Gln Tyr Ala Val Ile Ala Ile Val Pro Val Phe Cys Leu Met Gly
                165   170   175

Leu Leu Gly Ile Leu Val Cys Asn Leu Leu Lys Arg Lys Gly Tyr His
            180   185   190

Cys Thr Ala His Lys Glu Val Gly Pro Gly Pro Gly Gly Gly Gly Ser
        195   200   205

Gly Ile Asn Pro Ala Tyr Arg Thr Glu Asp Ala Asn Glu Asp Thr Ile
    210   215   220

Gly Val Leu Val Arg Leu Ile Thr Glu Lys Lys Glu Asn Ala Ala Ala
225   230   235   240

Leu Glu Glu Leu Leu Lys Glu Tyr His Ser Lys Gln Leu Val Gln Thr
            245   250   255

Ser His Arg Pro Val Ser Lys Leu Pro Pro Ala Pro Pro Asn Val Pro
        260   265   270

His Ile Cys Pro His Arg His His Leu His Thr Val Gln Gly Leu Ala
        275   280   285

Ser Leu Ser Gly Pro Cys Cys Ser Arg Cys Ser Gln Lys Lys Trp Pro
    290   295   300

Glu Val Leu Leu Ser Pro Glu Ala Val Ala Ala Thr Thr Pro Val Pro
305   310   315   320

Ser Leu Leu Pro Asn Pro Thr Arg Val Pro Lys Ala Gly Ala Lys Ala
            325   330   335

Gly Arg Gln Gly Glu Ile Thr Ile Leu Ser Val Gly Arg Phe Arg Val
        340   345   350

Ala Arg Ile Pro Glu Gln Arg Thr Ser Ser Met Val Ser Glu Val Lys
    355   360   365

Thr Ile Thr Glu Ala Gly Pro Ser Trp Gly Asp Leu Pro Asp Ser Pro
370   375   380

Gln Pro Gly Leu Pro Pro Glu Gln Gln Ala Leu Leu Gly Ser Gly Gly
385   390   395   400

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Ser Arg Thr Lys Trp Leu Lys Pro Pro Ala Glu Asn Lys Ala Glu Glu
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Asn Arg Tyr Val Val Arg Leu Ser Glu Ser Asn Leu Val Ile
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<222> (91)..(1398)

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tgagcgaagc tggtgctgcg ggccaggtca atg tca ctc cag ggc ctg atg atg    114
                                   Met Ser Leu Gln Gly Leu Met Met
                                    1               5

aag cgg acc ttg ctg tgc tgg ccc ctg tct tgc ctc ttt gtg ctg ctg    162
Lys Arg Thr Leu Leu Cys Trp Pro Leu Ser Cys Leu Phe Val Leu Leu
        10              15              20

ccc tgg cct ctg gcc act cca aca cca ata act cct tgg ctg tgt cca    210
Pro Trp Pro Leu Ala Thr Pro Thr Pro Ile Thr Pro Trp Leu Cys Pro
 25              30              35              40

cct ggc aaa gag cct gac cca gat cca gga cag ggc aca tta tgc aga    258
Pro Gly Lys Glu Pro Asp Pro Asp Pro Gly Gln Gly Thr Leu Cys Arg
                45              50              55

act tgc ccc cca gga acc ttt tca gcc tca tgg aac tcc tat cca tgc    306
Thr Cys Pro Pro Gly Thr Phe Ser Ala Ser Trp Asn Ser Tyr Pro Cys
                60              65              70

cag cct cat tac cga tgc agc ctt caa aag agg ctg gag gcc cag gct    354
Gln Pro His Tyr Arg Cys Ser Leu Gln Lys Arg Leu Glu Ala Gln Ala
            75              80              85

ggc aca gca act cat gat aca atg tgt gga gac tgc cag cat ggg tgg    402
Gly Thr Ala Thr His Asp Thr Met Cys Gly Asp Cys Gln His Gly Trp
        90              95              100

ttt ggg cca cag gga gtt cct cat gtt ccg tgt cag cca tgt tcc aag    450
Phe Gly Pro Gln Gly Val Pro His Val Pro Cys Gln Pro Cys Ser Lys
105             110             115             120

gca cct cca agt act ggt ggc tgt gat gag tca ggg cgg cgg ggc cgg    498
Ala Pro Pro Ser Thr Gly Gly Cys Asp Glu Ser Gly Arg Arg Gly Arg
                125             130             135
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cgt ggc gtc gaa gtg gca gca ggt acc agt agc aac ggt gaa cct cgg    546
Arg Gly Val Glu Val Ala Ala Gly Thr Ser Ser Asn Gly Glu Pro Arg
            140                 145                 150

cag ccc ggg aat ggc act cgg gca ggc ggt cct gag gag acg gct gcc    594
Gln Pro Gly Asn Gly Thr Arg Ala Gly Gly Pro Glu Glu Thr Ala Ala
            155                 160                 165

cag tat gca gtg att gcc atc gtt cct gtc ttt tgt ctc atg ggg ctt    642
Gln Tyr Ala Val Ile Ala Ile Val Pro Val Phe Cys Leu Met Gly Leu
        170                 175                 180

ctg ggc atc ctg gtg tgc aac ctg ctc aag cgg aag ggc tac cat tgc    690
Leu Gly Ile Leu Val Cys Asn Leu Leu Lys Arg Lys Gly Tyr His Cys
185                 190                 195                 200

aca gcc caa aag gaa gtt ggg ccc agc cct ggt gga gga ggc agc ggg    738
Thr Ala Gln Lys Glu Val Gly Pro Ser Pro Gly Gly Gly Gly Ser Gly
                205                 210                 215

att aat cct gcc tat agg act gaa gat gcc aac gag gac acc att gga    786
Ile Asn Pro Ala Tyr Arg Thr Glu Asp Ala Asn Glu Asp Thr Ile Gly
            220                 225                 230

gtc ctg gtg cgc ctg atc aca gag aag aaa gag aat gca gcg gcc ctg    834
Val Leu Val Arg Leu Ile Thr Glu Lys Lys Glu Asn Ala Ala Ala Leu
            235                 240                 245

gag gag ctg ttg aaa gaa tat cac agc aaa cag ctg gta cag aca agt    882
Glu Glu Leu Leu Lys Glu Tyr His Ser Lys Gln Leu Val Gln Thr Ser
            250                 255                 260

cac agg cct gta ccc agg ctg ctg ccg gcc tca ccc agc ata ccc cac    930
His Arg Pro Val Pro Arg Leu Leu Pro Ala Ser Pro Ser Ile Pro His
265                 270                 275                 280

atc tgc ccg cat cac cac cac ctg cac act gtg cag ggc ctg gcc tca    978
Ile Cys Pro His His His His Leu His Thr Val Gln Gly Leu Ala Ser
                285                 290                 295

ctc tct ggc ccc tgt tgc tcc cgt tgt agc cag aag tgg cca gag gtg   1026
Leu Ser Gly Pro Cys Cys Ser Arg Cys Ser Gln Lys Trp Pro Glu Val
                300                 305                 310

ctg ctg tct cct gag gca gca gct gcc acc act cct gct ccc acc ctt   1074
Leu Leu Ser Pro Glu Ala Ala Ala Ala Thr Thr Pro Ala Pro Thr Leu
            315                 320                 325

ctg cct act gca tcc agg gct ccc aag gct agt gcc aag cca gga cgt   1122
Leu Pro Thr Ala Ser Arg Ala Pro Lys Ala Ser Ala Lys Pro Gly Arg
        330                 335                 340

cag ggc gag att acc atc ttg tct gtg ggc agg ttc cgt gtg gct cgt   1170
Gln Gly Glu Ile Thr Ile Leu Ser Val Gly Arg Phe Arg Val Ala Arg
345                 350                 355                 360
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att cct gag cag cgg acc agt tca ttg tta tct gag gtg aag acc atc   1218
Ile Pro Glu Gln Arg Thr Ser Ser Leu Leu Ser Glu Val Lys Thr Ile
            365             370             375

acg gag gct ggg cct tca gag ggt gat ctc cct gac tcc cca cag cct   1266
Thr Glu Ala Gly Pro Ser Glu Gly Asp Leu Pro Asp Ser Pro Gln Pro
            380             385             390

ggt ttt ccc ccc gag cag cgg gca ctg ctg gga agt ggt ggg agc cat   1314
Gly Phe Pro Pro Glu Gln Arg Ala Leu Leu Gly Ser Gly Gly Ser His
            395             400             405

act aag tgg ttg aag ccc cca gca gag aac aaa gct gag gag aac cgc   1362
Thr Lys Trp Leu Lys Pro Pro Ala Glu Asn Lys Ala Glu Glu Asn Arg
        410             415             420

tat gtg gtc cgg cta agt gaa agc aac ctg gtc atc tgatgggctg        1408
Tyr Val Val Arg Leu Ser Glu Ser Asn Leu Val Ile
425             430             435
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tctagaatta gacactctgc cctgtcctgg gaggttctga aggcttcctg caggagggag 1468

agctgcagct gggactgagg accaagatgc aaaggccaag tcctggaggt gggaccgtcc 1528

gccccactga ggaggcagcc tgcggcacag cacgtgagca ggagatcaag agcccaccct 1588

atccctgcag tcccggttac ttccatgcag ggtgctgtaa ccctgtgcct gccctgaaca 1648

catcatagga gccctctgtc ccttagaggt ctggtttggt ggaggagtgg tatctgtacc 1708

tggccccaag cttgtgcctt gggaactagc cactcttgcc catgtcctgg accctggatg 1768

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gggaagagga gacaggcagg ctagggagtg cctgtggcct gtggttttca atgcccctgt 2248

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gcccatccca tttcttattt attgaaacct gctgttgccc tgcccctaca tctccagccc 2428

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<211> 436
<212> PRT
<213> Mus musculus

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 1               5                   10              '  15

Leu Ser Cys Leu Phe Val Leu Leu Pro Trp Pro Leu Ala Thr Pro Thr
            20                  25                  30

Pro Ile Thr Pro Trp Leu Cys Pro Pro Gly Lys Glu Pro Asp Pro Asp
            35                  40                  45

Pro Gly Gln Gly Thr Leu Cys Arg Thr Cys Pro Pro Gly Thr Phe Ser
    50                  55                  60

Ala Ser Trp Asn Ser Tyr Pro Cys Gln Pro His Tyr Arg Cys Ser Leu
65                  70                  75                  80

Gln Lys Arg Leu Glu Ala Gln Ala Gly Thr Ala Thr His Asp Thr Met
                85                  90                  95

Cys Gly Asp Cys Gln His Gly Trp Phe Gly Pro Gln Gly Val Pro His
            100                 105                 110

Val Pro Cys Gln Pro Cys Ser Lys Ala Pro Pro Ser Thr Gly Gly Cys
            115                 120                 125

Asp Glu Ser Gly Arg Arg Gly Arg Arg Gly Val Glu Val Ala Ala Gly
    130                 135                 140

Thr Ser Ser Asn Gly Glu Pro Arg Gln Pro Gly Asn Gly Thr Arg Ala
145                 150                 155                 160

Gly Gly Pro Glu Glu Thr Ala Ala Gln Tyr Ala Val Ile Ala Ile Val
                165                 170                 175

Pro Val Phe Cys Leu Met Gly Leu Leu Gly Ile Leu Val Cys Asn Leu
            180                 185                 190

Leu Lys Arg Lys Gly Tyr His Cys Thr Ala Gln Lys Glu Val Gly Pro
            195                 200                 205

Ser Pro Gly Gly Gly Gly Ser Gly Ile Asn Pro Ala Tyr Arg Thr Glu
    210                 215                 220

Asp Ala Asn Glu Asp Thr Ile Gly Val Leu Val Arg Leu Ile Thr Glu
225                 230                 235                 240
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Lys Lys Glu Asn Ala Ala Ala Leu Glu Glu Leu Leu Lys Glu Tyr His
            245             250             255

Ser Lys Gln Leu Val Gln Thr Ser His Arg Pro Val Pro Arg Leu Leu
            260             265             270

Pro Ala Ser Pro Ser Ile Pro His Ile Cys Pro His His His His Leu
            275             280             285

His Thr Val Gln Gly Leu Ala Ser Leu Ser Gly Pro Cys Cys Ser Arg
    290             295             300

Cys Ser Gln Lys Trp Pro Glu Val Leu Leu Ser Pro Glu Ala Ala Ala
305             310             315             320

Ala Thr Thr Pro Ala Pro Thr Leu Leu Pro Thr Ala Ser Arg Ala Pro
            325             330             335

Lys Ala Ser Ala Lys Pro Gly Arg Gln Gly Glu Ile Thr Ile Leu Ser
            340             345             350

Val Gly Arg Phe Arg Val Ala Arg Ile Pro Glu Gln Arg Thr Ser Ser
            355             360             365

Leu Leu Ser Glu Val Lys Thr Ile Thr Glu Ala Gly Pro Ser Glu Gly
    370             375             380

Asp Leu Pro Asp Ser Pro Gln Pro Gly Phe Pro Pro Glu Gln Arg Ala
385             390             395             400

Leu Leu Gly Ser Gly Gly Ser His Thr Lys Trp Leu Lys Pro Pro Ala
            405             410             415

Glu Asn Lys Ala Glu Glu Asn Arg Tyr Val Val Arg Leu Ser Glu Ser
            420             425             430

Asn Leu Val Ile
        435
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<213> Homo sapiens

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acattatgca ggccctgccc cccaggcacc ttctcagctg catggggctc cagcccatgc 180

cagccccatg cccgttgcag cctttggagg aggctggagg cccaggtggg catggcaact 240

cgagatacac tctgtggaga ctgctggcct gggtaagcca aagggagtgc ggggagggct 300
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cctggctggg tgaccaggac tctggatcct ggggccccag ccttattgta ccctgagcag 360

gcctcattct tcccatctgt gaaatgggat ggggcaggac cacggagggt gcctggtagg 420

aaggaatcca gcctctccta aggatagtgt ttggggaaac ttctgggcct cagtggtatc 480

tt   482

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<212> PRT
<213> Homo sapiens

<400> 6
Asp Pro Gly Gln Gly Thr Leu Cys Arg Pro Cys Pro Pro Gly Thr Phe
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Ser Ala Ala Trp Gly Ser Ser Pro Cys Gln Pro His Ala Arg Cys Ser
            20   25   30

Leu Trp Arg Arg Leu Glu Ala Gln Val Gly Met Ala Thr Arg Asp Thr
        35   40   45

Leu Cys Gly Asp Cys Trp Pro Gly
    50   55

<210> 7
<211> 91
<212> PRT
<213> Homo sapiens

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Leu Ser Trp Glu Gly Pro Glu Gly Gln Gly Gln Ser Pro Val Pro Gly
 1   5   10   15

Pro Gln Gly Ser Ser Gly Leu Ala Pro Gly His Ala Leu Thr Leu Tyr
            20   25   30

Leu Pro Gln Asp Pro Gly Gln Gly Thr Leu Cys Arg Pro Cys Pro Pro
        35   40   45

Gly Thr Phe Ser Ala Ala Trp Gly Ser Ser Pro Cys Gln Pro His Ala
    50   55   60

Arg Cys Ser Leu Trp Arg Arg Leu Glu Ala Gln Val Gly Met Ala Thr
65   70   75   80

Arg Asp Thr Leu Cys Gly Asp Cys Trp Pro Gly
                85   90

<210> 8

- 12 -

<211> 120
<212> PRT
<213> Homo sapiens

<400> 8
Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr
 1   5   10   15

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe
                20   25   30

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg
            35   40   45

Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys
        50   55   60

Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys
65   70   75   80

Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr
                85   90   95

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
            100   105   110

Phe Ala Val Pro Thr Lys Phe Thr
            115   120

<210> 9
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer
      2374-51

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<210> 10
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<212> DNA
<213> Artificial Sequence

<220>
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<212> DNA
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<223> Description of Artificial Sequence:PCR primer
      2374-53

<400> 13
cccaggccag cagtctccac ag                                        22


<210> 14
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer
      1019-06

<400> 14
gctctaatac gactcactat aggg                                      24


<210> 15
<211> 29
<212> DNA
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- 14 -

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer
      1916-82

<400> 15
catgattacg ccaagctcta atacgactc   29

<210> 16
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer
      1340-35

<400> 16
cccagtcacg acgttgtaaa acg   23

<210> 17
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer
      1019-05

<400> 17
tgaatttagg tgacactata gaagag   26

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer
      1019-05

<400> 18
gcccgttgca gcctttggag   20

<210> 19
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

- 15 -

<223> Description of Artificial Sequence: PCR primer

<400> 19
tccaccaccc tgttgctgta g   21

<210> 20
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 20
gaccacacag tccatgccat cact   24

<210> 21
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 21
ccatcgatgg ctgagcagca ggtgtggaca   30

<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 22
tggcgatgac ggtgacctgg gcgg   24

<210> 23
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 23
Ser Thr Thr Leu Trp Gln Cys Pro Pro Gly Glu Glu
 1   5   10

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<210> 24
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 24
Gly Val Glu Val Ala Ala Gly Ala Ser Ser Gly Gly Glu Thr
  1               5                  10


<210> 25
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Furin cleavage
      site

<400> 25
Arg Arg Ala Arg Arg Gly Val Glu Val
  1               5


<210> 26
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: FLAG epitope
      sequence

<400> 26
Met Asp Tyr Lys Asp Asp Asp Asp Lys
  1               5


<210> 27
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 27
Gly Arg Lys Lys Lys Arg Arg Gln Arg Arg Arg
  1               5                  10


<210> 28
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<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide

<400> 28
Gly Gly Gly Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
 1   5   10   15

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C12N15/12   C12N15/62   C12N5/10   C07K14/705   C07K16/28
         G01N33/50   G01N33/566   C12Q1/68   A01K67/027   A61K31/70
         A61K38/17   A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C12N   C07K   G01N   C12Q   A01K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL SEQUENCES 'Online! Accession No. AI223336, 28 October 1998 (1998-10-28) STRAUSBERG R.: "EST; H. sapiens cDNA clone IMAGE:1840621" XP002162732 nt 16-421 are complementary to nt 2233-2638 of seq. ID 1. ---	1-13, 15-26,57
P,X	DATABASE EMBL SEQUENCES 'Online! Accession No. Q9N092, 1 October 2000 (2000-10-01) OSADA N.: "Unnamed protein product from Macaca fascicularis" XP002162733 compare with seq. ID 2. ---	1-69, 71-82

-/--

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 2001	26/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 02568 A (CHIRON CORP ;HYSEQ INC (US)) 11 January 2001 (2001-01-11) compare nt 1969-2336 of seq. ID 1 with nt 10-369 of seq. 442 of WO0102568 ---	1-13, 15-26,57
A	WO 97 23614 A (AMGEN INC ;LACEY DAVID L (US); BOYLE WILLIAM J (US); CALZONE FRANK) 3 July 1997 (1997-07-03) the whole document ---	1-69, 71-82
A	SMITH C A ET AL: "THE TNF RECEPTOR SUPERFAMILY OF CELLULAR AND VIRAL PROTEINS: ACTIVATION, COSTIMULATION, AND DEATH" CELL, US, CELL PRESS,, vol. 76, 25 March 1994 (1994-03-25), pages 959-962, XP002029050 ISSN: 0092-8674 the whole document ---	1-69, 71-82
A	ADAMS M D ET AL: "COMPEMENTARY DNA SEQUENCING: EXPRESSED SEQUENCE TAGS AND HUMAN GENOME PROJECT" SCIENCE, US, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 252, no. 5013, 21 June 1991 (1991-06-21), pages 1651-1656, XP000645049 ISSN: 0036-8075 the whole document -----	11,26

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 70

1. Claim 17 refers to the orthologue encoded by the polynucleotide of
seq. 6. This sequence is a fragment of the human protein decribed, which
is inconsistent with the claim. Claim 70 is assumed to be dependent on
claim 69, not 70. Claim 79 is dependent on the (inexistent) claim 84.
It is assumed to depend on claim 77. Claims 81-82 are dependent on
(inexistent) claims 86-87. It is assumed that they are dependent on
claims 79-80.

2. Claim 70 relates to a binding partner of the TGFR/OPG identified by
the two-hybrid screening system. Claims 32, 41, 43-46, 72-73 relate, at
least in part, to selective binding agents and/or antagonists of the
polypeptide claimed, but without providing a satisfactory technical
characterization or being limited to elements based on clearly
identifiable properties of the TNFR/OPG polypeptide or of the
corresponding polynucleotides, such as, e.g., antibodies or antisense
oligonucleotides. In consequence, said claims are ambiguous and vague.
Moreover, no such compounds are described in the application, so that the
claims lack the support required by Art. 5 and 6 PCT. No meaningful
search can be carried out for such purely speculative claims, the wording
of which is, in fact, a mere list of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of
claims, relating to inventions in respect of which no international
search report has been established need not be the subject of an
international preliminary examination (Rule 66.1(e) PCT). The applicant
is advised that the EPO policy when acting as an International
Preliminary Examining Authority is normally not to carry out a
preliminary examination on matter which has not been searched. This is
the case irrespective of whether or not the claims are amended following
receipt of the search report or during any Chapter II procedure.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0102568	A	11-01-2001	NONE		
WO 9723614	A	03-07-1997	AU	710587 B	23-09-1999
			AU	1468697 A	17-07-1997
			BG	101813 A	30-09-1998
			CA	2210467 A	03-07-1997
			CN	1182452 A	20-05-1998
			CZ	9702538 A	17-03-1999
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			FR	2742767 A	27-06-1997
			GB	2312899 A,B	12-11-1997
			HU	9801122 A	28-08-1998
			JP	11503616 T	30-03-1999
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			NZ	326579 A	28-01-1999
			PL	321938 A	05-01-1998
			SK	110797 A	12-07-1999
			TR	970550 A	21-07-1997
			US	6015938 A	18-01-2000